

Proximal Refractive Index Gradients in the Crystalline Cones of Crustacean Compound Eyes

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1983

FINAGLE's RULES

1. *To study a subject best, understand it thoroughly before you start.*
2. *Always keep a record of data - it indicates you've been working.*
3. *Always draw your curves, then plot your reading.*
4. *In case of doubt, make it sound convincing.*
5. *Experiments should be reproducible - they should all fail in the same way.*
6. *Do not believe in miracles - rely on them.*

MURPHY

PROXIMAL REFRACTIVE INDEX GRADIENTS IN THE CRYSTALLINE
CONES OF CRUSTACEAN COMPOUND EYES

Doctoral Thesis

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Fil.kand Dan-Eric Nilsson

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Abstract Numerous pelagic or planktonic crustaceans have remarkably transparent compound eyes. From observations of the pseudopupil and from morphology, it was obvious that these eyes are of the apposition type, thus having optically isolated ommatidia. Usually, optical isolation is achieved by extensive interommatidial pigment screens, but in the transparent eyes, the exceptionally long crystalline cones are unscreened. This raised the question of how optical isolation can be maintained with a seemingly insufficient pigment screen. The problem was approached by an optical analysis of the crystalline cones. For this purpose a new method was developed for interferometric determination of refractive index gradients in intact objects. With this method, the refractive index distribution in entire crystalline cones could be accurately determined and analysed with ray-tracing in two-dimensional models. This was first applied to the transparent compound eye of the pelagic amphipod <i>Hyperia galba</i> and it was found that the long crystalline cones have a gradient of increasing refractive index towards the proximal tip (proximal gradient). This gradient rejects rays entering from adjacent facets and thereby constitutes a substitute for screening pigment between the cones. The biological significance of this system was interpreted as a way of reducing the pigment screen for achieving a maximally transparent and thus well camouflaged eye. Continued investigations on other crustaceans (the cladoceran <i>Leptodora kindtii</i>, the isopod <i>Astacilla longicornis</i> and the larvae of decapods and some euphausiids) established the connection between proximal gradients and transparent eyes. Moreover, transparent eyes were shown to be pre-adapted to superposition optics, thus revealing a possible evolutionary link between apposition and superposition eyes in crustaceans. Finally, proximal gradients were demonstrated in the reflecting superposition eye of the decapod prawn <i>Macrobrachium rosenbergii</i>, where they probably aid in adaptation from dark to light conditions.		
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PAPERS (which will be referred to by their roman numerals):

I.	Nilsson, D.-E. The transparent compound eye of <i>Hyperia</i> (Crustacea): Examination with a new method for analysis of refractive index gradients. J. Comp. Physiol. 147: 339-349 (1982)	21
II.	Nilsson, D.-E., Odselius, R. & Elofsson, R. The compound eye of <i>Leptodora kindtii</i> (Cladocera): An adaptation to planktonic life. Cell Tissue Res. (in press)	33
III.	Nilsson, D.-E. & Nilsson, H. L. Eye camouflage in the isopod crustacean <i>Astacilla longicornis</i> (Sowerby). J. Exp. Mar. Biol. Ecol. 68 (in press)	43
IV.	Nilsson, D.-E. The evolutionary links between apposition and superposition optics in crustacean eyes. (submitted for publication)	49
V.	Nilsson, D.-E. Refractive index gradients subserve optical isolation in a light-adapted reflecting superposition eye. J. Exp. Zool. (in press)	57

1. Presentation

This thesis describes the mechanisms and occurrence of proximal refractive index gradients in the crystalline cones of crustacean compound eyes. The existence of such gradients is a recent discovery that was made independently by Dr. M. Land at the University of Sussex (1981a) and myself (I). On exploring the subject I found that proximal gradients subserve important optical mechanisms in many crustacean eyes.

These findings were made possible by the development of a method for non-destructive determination of refractive index gradients. This method is outlined briefly in paper I and a detailed description is published separately (Nilsson et al. 1983).

2. General introduction

Compound eyes are the main visual organs in insects and crustaceans, but they are also reported in chelicerates (*Limulus*, Grenacher 1879), myriapods (*Scutigera*, Hanström 1934) and the extinct trilobites (Lindström 1900). In non-arthropods, compound eyes are only known from a few annelid worms (Hesse 1899) and a few molluscan bivalves (Patten 1886).

Wherever compound eyes are found, they are of either the apposition or superposition type. The first account of the apposition principle dates back to Müller (1826) who formulated the mosaic theory. A more thorough analysis was provided by Sigmund Exner in his classical 1891 monograph which, after almost 100 years, still remains one of the most complete accounts of optics in compound eyes. The most important of Exner's contributions is the definition of the two fundamental principles of image formation, apposition and superposition. In the apposition eye, the ommatidia are optically isolated i.e. each rhabdom, which acts as a light guide, receives light only from its 'own' lens. In a superposition eye, the lens system of many ommatidia (mainly the crystalline cones) form a superimposed image on a layer of rhabdoms in the depth of the eye. A comprehensive presentation of this optical principle is given in paper IV.

Exner's theories have been challenged several times (e.g. Nunnemacher 1959; Kuiper 1962; Burt & Catton 1962; Horridge 1969, 1971). However, later investigations have provided evidence that Exner was right about the apposition and superposition principles (Varela & Wiitanen 1970; Horridge et al. 1972; Cleary et al. 1977). Although this seems to have brought us back to what was known a century ago, two fundamental advances have been made in compound eye optics since Exner. These are the discovery of neural superposition eyes (a refinement of optical apposition) in flies and some other insects (Kirschfeld 1967; Ioannides & Horridge 1975) and the reflecting superposition optics in the eyes of decapod crustaceans (Vogt 1975, 1977; Land 1976).

Refraction by gradients (i.e. a continuous variation of the refractive index) in compound eyes was proposed already by Exner (1891), but the actual proof of the existence of

such gradients was first reported in 1969 by Seitz. Since then, a continuously increasing number of species have been proved to possess graded index lenses in the cornea and the crystalline cones (Kunze & Hausen 1971; Hausen 1973; Horridge et al. 1972; Ioannides & Horridge 1975; Vogt 1974, 1977; Land 1979, 1981a; Land & Burton 1979; Caveney & McIntyre 1981; Bryceson 1981; Nilsson & Odselius 1983; I; II; III; IV; V).

Although it is easy to reveal the existence of refractive index gradients, it has been a serious difficulty to obtain quantitative measurements. This difficulty has now been overcome by a new method which permits convenient and highly accurate determinations of refractive index gradients in intact live crystalline cones (I; Nilsson et al. 1983).

With this new method available I decided to tackle an optical enigma in the compound eye of the amphipod crustacean *Hyperia*. On a morphological basis and due to the small black pseudopupil (see Franceschini 1975), it could be deduced that *Hyperia* has an apposition eye (as amphipods in general). Unlike other apposition eyes, the crystalline cones are extremely long and devoid of their usual pigment screen, which make the eye almost transparent. The enigma was how optical isolation could be maintained between these long and slender cones. The problem was solved by the discovery of refractive index gradients in the proximal part of the crystalline cones, which acts as a substitute for screening pigment (I).

Continued investigations revealed the occurrence of transparent compound eyes with proximal gradients (of two types) in several crustacean groups (II; III; IV). Hence, the principle of a new type of apposition eye could be established. It was also shown that transparent apposition eyes may be the functional link in the evolution of superposition eyes in crustaceans (IV). Finally, proximal gradients were found to be present in the reflecting superposition eye of a decapod prawn (V).

3. Analysis of refractive index gradients

Preparations for interferometry

Determinations of refractive index gradients in crystalline cones were made interferometrically on isolated cones (I-V). Due to the problem of isolating the cones without damaging them, different approaches were tried. To begin with, eyes were fixed (3% glutaraldehyde for 15 min to harden the tissue) to obtain an estimate of the shape of intact cones. This was followed by preparations of fresh eyes in balanced salt solutions (Nilsson & Odselius 1981) which resulted in a varying success-rate, depending on the species and eye region. For *Leptodora* (II), *Astacilla* (III) and the *Thysanoessa* larva (IV) this was relatively easy and successful preparations were obtained most times. For *Hyperia* (I) and the *Macrobrachium* larva (IV) the success-rates were extremely low, necessitating up to 100 preparations to obtain a few intact cones. For adult *Macrobrachium* (V), however, the softness and

instability of fresh cones prevented any successful isolation. The instability of crystalline cones in reflecting superposition eyes has been documented earlier (Land 1976; Vogt 1980). Fortunately, as the pseudopupil of adult *Macrobrachium* was not affected by fixation, fixed eyes could be used as a reasonable model of the fresh eye.

Interferometry

The relation between phase shift and refractive index was used to determine the refractive index of the crystalline cones. Phase-shift measurements were made interferometrically with a Jamin-Lebedeff microinterferometer (interference microscope), employing transverse interferometry. Thus, the interference patterns were recorded from intact cones with their longitudinal axes perpendicular to the optical axis of the microscope. The interference patterns were analysed from photographs which eliminated any effects of aging of the preparations.

Two different techniques were used to obtain phase-shift profiles with a maximum number of determined points:

- (1) analysis of the interference colours (I; II; III),
- (2) fringe-shift, using de-Sénarmont compensation and monochromatic light (IV; V). Both techniques yielded phase-shift recordings at $1/4 - 1/6$ wavelength intervals.

The iterative method

The interference patterns were measured as phase-shift profiles (phase shift as a function of radial distance). Assuming rotational symmetry of the cones, refractive index could be expressed as a function of the radial distance. Thus, the task was to convert phase-shift profiles to refractive index profiles. The phase shift of a ray is a result of the path distance and the refractive index. In the transverse interferograms, the ray path is curved by the object and this curvature, which is needed to calculate the refractive index, can be determined only if the refractive index is already known. To solve this problem, an iterative method was designed. This method is outlined briefly in paper I and an improved version is described in detail by Nilsson et al. (1983). The improvement is a correction for different path distances of the reference- and object-beams in the interference microscope (see Kahl & Mylin 1965). The improved method was used in papers III and IV but not in papers I, II and V. However, the original and improved method gave similar results and the improvement has little or no effect on the subsequent ray-tracings in the cone models. Theoretically, however, the improved method enables the accuracy to approach the resolution of the microscope objective.

Ray-tracing

The refractive index profiles were combined to create two-dimensional refractive index maps of the crystalline cones. These maps were analysed by ray-tracing. In the case of radial gradients (I; II; IV) the curved ray paths had to be

taken into account. Doing this with manual ray-tracings is time-consuming and yields low accuracy. Computerized calculations of the ray paths are convenient and highly accurate (Meggett & Meyer-Rochow 1975; Cleary et al. 1977), but this entails the fitting of a computer program to the refractive index map of the actual cone. To account for the many differently shaped crystalline cones described in this thesis, a compromise between the computerized and manual techniques was used. Plotting of a ray trajectory was made by hand, stepwise in a co-ordinate system. For each step, a programmable calculator was fed with the local values of the refractive index gradient. The calculator was used to currently store and compute new co-ordinates, thus eliminating any effects of imprecise manual plotting.

4. Theory of optically isolating mechanisms

In this thesis, refractive index gradients in the crystalline cones are described for five species. These gradients were found to maintain optical isolation, thus substituting screening pigment. Two types of gradients could be distinguished: (1) an axial gradient with increasing index values towards the proximal part of the cone (III; IV; V), (2) a combination of axial and radial gradients with a maximum index value along the optical axis in the proximal part of the cone (I; II; IV). Based on the present data, it is now possible to work out optical theories for the two types of gradients.

Refraction at the cone border

The crystalline cones without radial gradients can be described by a simple model consisting of a cone with a cut tip. The refractive index of the cone is n_1 and it is surrounded by a homogeneous fluid with refractive index n_0 . The cone tapers with an angle δ (half the cone angle, Fig. 1a) and the diameter of the cut tip, d (proximal tip) corresponds to the diameter of the distal rhabdom tip. The condition for optical isolation is that no rays entering through the sides of the cone should reach the rhabdom. In a plane containing the axis, a ray entering parallel to the side must be refracted through an angle $>90^\circ - \Psi$ (where Ψ is the angle between the side normal and the refracted ray) to project outside the rhabdom (skew rays neglected). The task is thus to find $n_1 = f(n_0, \delta, d)$ for the limit refraction $90^\circ - \Psi$ at the axial distance p .

From the geometry in Fig. 1a we find,

$$p = A \cot \delta \quad (1)$$

$$p = (d + A) \tan(\Psi - \delta) \quad (2)$$

$$A = p \tan \delta \quad (3)$$

Combining Eqs. (1) and (2) gives,

$$\frac{A \cot \delta}{d + A} = \tan(\Psi - \delta) \quad (4)$$

and hence,

$$\Psi - \delta = \arctan\left(\frac{A \cot \delta}{d + A}\right) \quad (5)$$

Introducing Eq. (3) leads to,

$$\Psi = \delta + \arctan\left(\frac{p \tan \delta \cot \delta}{d + p \tan \delta}\right) \quad (6)$$

which reduces to,

$$\Psi = \delta + \arctan\left(\frac{1}{(d/p) + \tan \delta}\right) \quad (7)$$

Using Snell's law we finally get,

$$n_1 = \frac{n_0}{\sin\left[\delta + \arctan\left(\frac{1}{(d/p) + \tan \delta}\right)^{-1}\right]} \quad (8)$$

We can now use Eq. (8) to calculate the minimum gradient required for optical isolation (Fig. 1b). In the proximal part, the minimum refractive index takes on values that

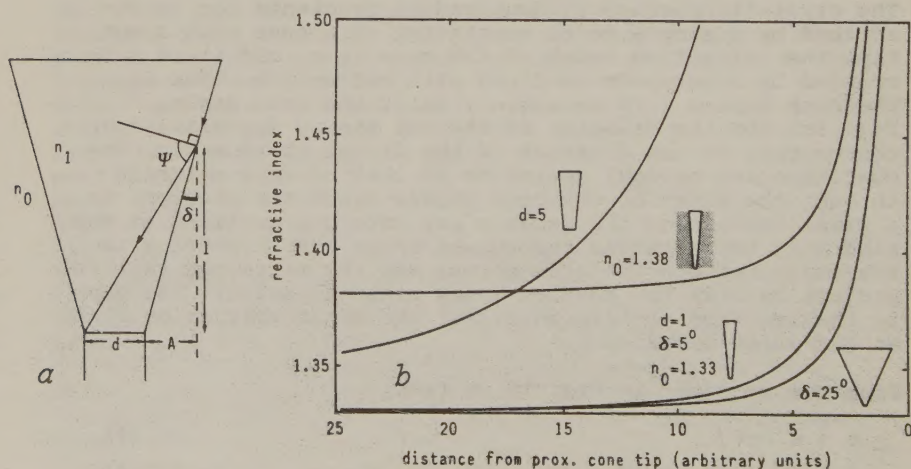


Fig. 1. a, calculation geometry for Eqs. 1-3. b, minimum refractive index gradients for optical isolation, calculated by Eq. 8 for four hypothetical cases. The second graph from the bottom represents a design similar to the actual cases (III; IV). The other three graphs show the impact of changing the three variables, d , n_0 , and δ individually.

cannot be realized in biological tissues. Therefore, to maintain optical isolation, the proximal tip must be shielded by pigment. By changing the values of n_0 , δ , and d it is possible to estimate the influence of these parameters on the required design. It can be seen in fig. 1b that the cone taper angle, δ , and the surrounding refractive index, n_0 , has little effect on the required distal extension of the pigment screen. However, the diameter of the proximal cone tip (distal rhabdom tip) is important and a small diameter is required to reduce the extension of the pigment screen. This is a serious limitation to transparent design because a wide acceptance angle can be achieved either by a short focal length or a wide rhabdom (Pask & Snyder 1975) and both these factors tend to reduce the

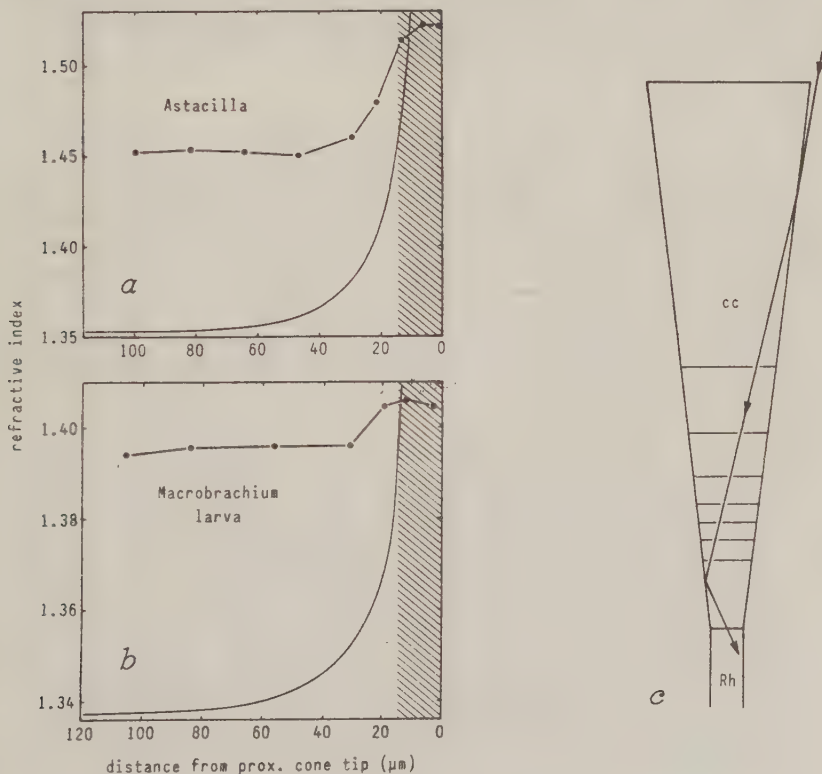


Fig. 2. a and b, measured axial gradients (dots) in crystalline cones of *Astacilla* and larva of *Macrobrachium* compared with their theoretical minimum gradients. Striped pattern indicates the extension of the pigment screen. c, illustration of total internal reflections that will occur if the gradient is too steep. cc, crystalline cone; Rh, rhabdom.

length of the transparent part of the ommatidium. Consequently, this optical principle would constitute substantially improved camouflage only in relatively large eyes where the acceptance angles are small.

Comparing the theoretical minimum gradient with the actual gradients found in *Astacilla* (III) and the *Macrobrachium* larva (IV), discrepancies can be noted (Fig. 2a, b). In the most proximal part of the cones, the gradient slope declines to form a plateau, which is explained by the pigment screen taking over the function of the gradient in this part. Distally in the crystalline cones, the actual refractive index is significantly higher than the minimum gradient. There are at least three possible reasons for this discrepancy.

(1) If total internal reflection of side-entering rays is to be avoided, the gradient cannot be too steep (Fig. 2c). (2) Side-entering skew rays would possibly need a stronger deflection to be rejected. (3) The higher refractive index that would theoretically be required in smaller eyes may partly remain during growth.

Afocal systems

In *Hyperia* (I), *Leptodora* (II) and the *Thysanoessa* larva (IV), transparent eyes have been realized with another type of proximal gradient in the crystalline cones which is characterized by both axial and radial refractive index gradients. The axial gradients are similar to those found in the previous type of system, which makes it tempting to assume a modification of the same principle. However, to account for the radial gradients, quite another theory must be deduced.

Due to the radial variation of the refractive index, the proximal gradient can be considered as a second lens which forms an image (c.i.) of the main lens (cornea or distal gradient). In the model in fig. 3a this image is focused well above the distal tip of the rhabdom. If the diameter of the proximal gradient at the image level (c.i. level) corresponds to the diameter of the imaged main lens, then all rays entering from any of the adjacent main lenses will emerge laterally from the gradient. Thus, optical isolation will be achieved by a mechanism fundamentally different from the cone border refraction described in the previous section.

As a consequence of the imaging properties of the proximal gradient, parallel rays entering the eye (as from a distant source) will be focused distal to the c.i. level and thus, far distal to the rhabdom (Fig. 3b). For this inverted image of the distant source, f.p.1, the proximal part of the gradient will act as a lens-cylinder and form a second erect image (f.p.2). Depending on the length of this lens-cylinder, the rays can enter the rhabdom in any focal condition (Fig. 3b). Due to the high numeric aperture of the lens-cylinder, a rhabdom connected to the cone at a focal plane (f.p.) would need an extremely high refractive index to function as a homogeneous light guide. It would be better if the rhabdom was connected at one of the afocal planes (a.p.). Such an arrangement would constitute an afocal apposition system where the acceptance angle is de-

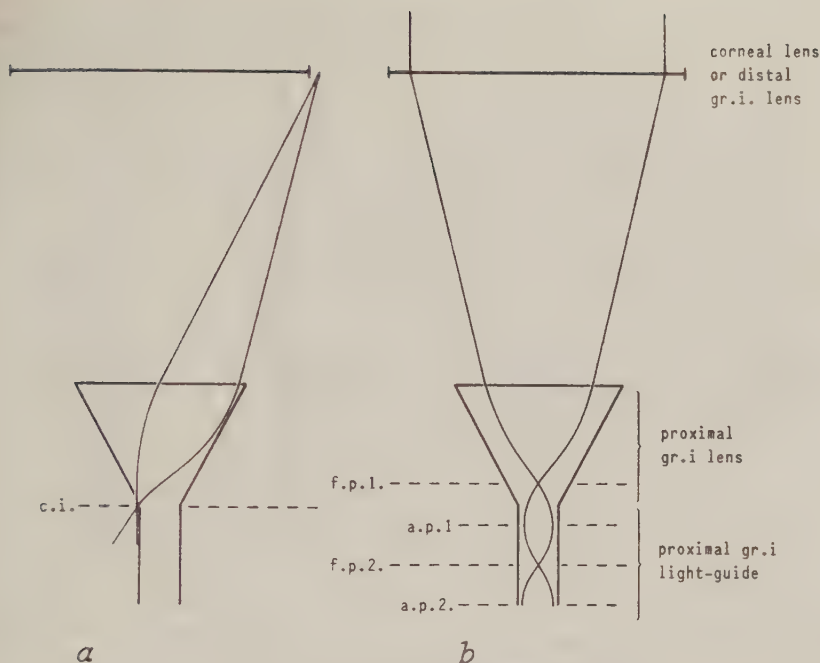


Fig. 3. The afocal principle for optical isolation. *a*, imaging of the distal lens array. *b*, focusing of a distant source.

terminated by the angles for which the rhabdom will act as a light guide.

To conclude, the system outlined above comprises the following functional elements: (1) a main distal lens made up of the cornea or a graded index (gr.i.) lens distally in the cone, (2) a proximal gr.i. lens with the capacity to form an image of the cornea, (3) a proximal gr.i. light-guide which propagates light to the rhabdom. To achieve optical isolation, only the proximal light-guide requires a pigment screen.

The above theory may seem very hypothetical but the actual ray paths demonstrated in papers I, II and IV, and fig. 4 closely fit this theory. In the *Thysanoessa* larva and probably also in *Hyperia*, the rhabdom is connected approximately at the second afocal plane (a.p.2). In *Leptodora*, the gr.i. light guide is short and not obvious, and the rhabdom is connected approximately at the first afocal plane (a.p.1).

Thus, I have found two fundamentally different mechanisms for maintaining optical isolation with a reduced pigment screen. The afocal system is by far the most complicated one but it has two features that make it superior to the cone-border-refraction system: (1) the pigment screen

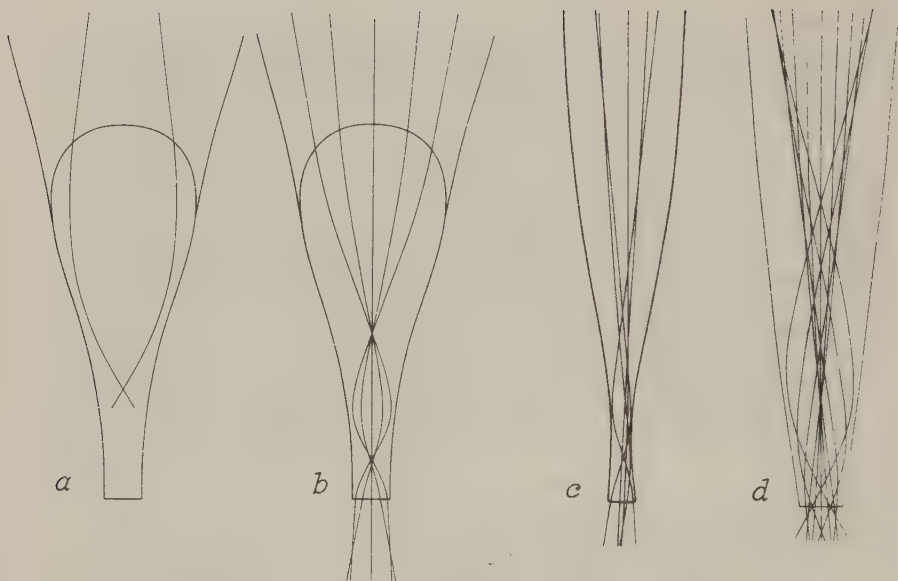


Fig. 4. Ray-tracing in proximal gradients; *a* and *b*, *Thysanoessa* larva; *c*, *Hyperia*; *d*, *Leptodora*. *a* shows the position of the c.i. level. *b*, *c* and *d* are ray paths from a distant source (parallel rays entering the cornea). The ray path in *b* is partly idealized because the corneal lens is taken into account as an aberration-free lens (only the focal length was measured). In *c* and *d*, the long focal length of the distal gradient (main lens) results in obvious aberrations which may partly be due to insufficient accuracy in tracing and interferometric analysis. Consequently, much of the difference in focal quality between *b*, *c* and *d* is probably artificial.

can be confined to a proximal level even in very small eyes (wide acceptance angles), (2) the system is not impaired by total internal reflections or skew rays.

5. The occurrence of proximal gradients

The huge planktonic biomass supports numerous predators, many of which are visually guided (e.g. fish). Protection by camouflage is therefore the most probable explanation for the remarkable transparency of animals in marine or limnic plankton. However, visual organs require dark screening pigment to achieve directional discrimination of the photoreceptors. As a result, the eyes are often the only pigmented parts in planktonic crustaceans. In this respect it is not surprising to find optical mechanisms in compound eyes that enable a substantial reduction of the pigment screen. Such mechanisms have now been found in several planktonic crustaceans as well as in crustaceans that are not planktonic or do not have transparent eyes. A survey

of the occurrence of pigment-reducing mechanisms is presented in the sections below.

Transparent apposition eyes

The first discovery of a transparent compound eye was made by Claus as early as 1879. He described the peculiar dorsal eye of the pelagic hyperiid amphipod *Phronima sedentaria*, in which the crystalline cones possess extremely elongated light-guides. Recently, these eyes have been studied anatomically (Ball 1977) and optically (Land 1981a), but the optically isolating mechanisms have not yet been investigated in this species. Hence, it is at present not possible to determine whether they employ cone border refraction or an afocal system. Neither can a combination of the two mechanisms nor a new one be excluded. Another hyperiid amphipod, *Hyperia* (I), seems to employ the afocal principle for optical isolation but cone border refraction may have a supporting role. On a structural basis it seems probable that the *Hyperia*-type of eye is typical for the hyperiid family (Meyer-Rochow 1978; Hallberg et al. 1980; Land 1981a; I).

With respect to planktonic life and eye type, the cladoceran superfam. Onychopoda constitutes a remarkable case of convergent development with the hyperiids. One member of this cladoceran group, *Leptodora* (II), was found to have an optical system almost identical to that of *Hyperia*. Judging from morphology, similar eyes are present in other onychopods, such as the marine genera *Podon* and *Evadne* and the limnic *Bythotrephes* (Milz 1899; own observations).

Hyperiids and onychopods are exclusively planktonic (or pelagic) but some exceptions occur regarding transparent eyes, viz. the hyperiid *Vibilia* (own observation) and the onychopod *Polyphemus* (Nilsson & Odselius 1983; Odselius & Nilsson 1983), both of which have screening pigment around the crystalline cones.

Transparent apposition eyes have also been found to be generally occurring in the planktonic larvae of euphausiids and decapods (IV). Euphausiid larvae probably have a pure afocal system, whereas decapod larvae maintain optical isolation by the cone border refraction principle. It was demonstrated that the apposition eyes of these larvae are potential superposition eyes (IV). It can now be concluded that this is a general feature of transparent eyes (Fig. 5). Thus, the cone border refraction principle provides the functional basis for the reflecting superposition eye, and the transparent afocal system is a potential refracting superposition system. Since reflecting and refracting superposition eyes are known from adult decapods and euphausiids respectively, it seemed fair to propose that transparent eyes actually were the evolutionary origin of superposition eyes in crustaceans (IV).

In one isolated case, a transparent apposition eye was found in an animal that is neither planktonic nor transparent. This is the isopod *Astacilla* (III), which has an eye based on the cone border refraction principle. *Astacilla* is camouflaged by mimicking the sea-whip on which it lives and it is probable that the transparent optics help to conceal its relatively large, bulging eyes.

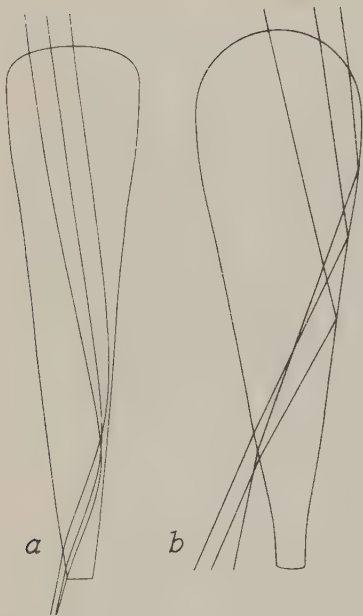


Fig. 5. Ray-tracings demonstrating unutilized superposition rays in *a*, *Leptodora* and *b*, *Astacilla*. Note that superposition rays are formed by refraction in *a* and by reflection in *b*. See also fig. 7b in paper I and fig. 2 in paper IV.

The nauplius eyes of some cyclopoid copepods

With the present knowledge on transparent compound eyes, interesting parallels could be drawn with the aberrant nauplius eyes of some planktonic copepods, viz. *Copilia*, *Corycaeus*, and *Sapphirina*. The nauplius eyes of these species form a pair of forward-directed units which perform rapid scanning movements (Gregory et al. 1964). Each of these units comprises a large distal lens and a small proximal lens connected to a receptor unit (Exner 1891; Vaissière 1961; Elofsson 1966; Land 1981b; Fig. 6). Screening pigment encompasses the receptor unit and part of the proximal lens, but the wide space between the two lenses is unscreened in conformity with the overall transparency of these animals. This optical design is almost identical with that of a single ommatidium in the larval compound eye of *Thysanoessa* (IV). Thus, it seems that the same optical principle has been derived in two non-homologous visual organs.

Reflecting superposition eyes

The details of reflecting superposition optics were worked out by Vogt (1980). In the crayfish *Astacus* he found a decreasing refractive index towards the proximal part of the crystalline cone tail. A different pattern was found in the

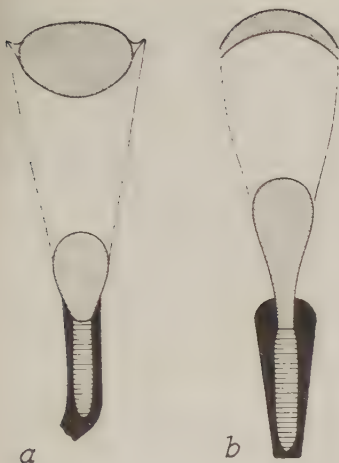


Fig. 6. Semischematic drawings of *a*, a lateral cup of the nauplius eye of the copepod *Sapphirina* (drawn from a histological section), and *b*, an ommatidium of the larval compound eye of the euphausiid *Thysanoessa* (IV). Receptor units (rhabdoms) are indicated by striped patterns and screening pigment by black areas.

reflecting superposition eye of the prawn *Macrobrachium* (V). In this species, the distal part of the cone tail showed the same type of gradient as that of *Astacus*, but the proximal part displayed a pronounced increase in refractive index. This proximal gradient rejects superposition rays if the proximal cone tips are surrounded by screening pigment (as they are in the light-adapted state). As a result of this, the eye can convert from superposition to apposition optics during light adaptation, with a minimum of pigment migrations. This was interpreted as a mechanism to decrease the time required for light adaptation (V).

The discovery of this mechanism in the adult *Macrobrachium* was perhaps not surprising, since the same basic principle was found in the larval apposition eye of the same species (IV). With respect to refractive index in the cone tails of reflecting superposition eyes, *Astacus* and *Macrobrachium* are the only species investigated and data from other species would be required to determine whether proximal gradients is a common feature of reflecting superposition eyes.

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The Transparent Compound Eye of *Hyperia* (Crustacea): Examination with a New Method for Analysis of Refractive Index Gradients

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Summary. The optical system in the compound eye of the amphipod *Hyperia galba* (Crustacea) was examined. The cornea is flat and lacks focusing properties. The crystalline cones are extremely long (up to 600 μm) and not shielded by screening pigment. Thus, the eye is transparent except for the dark retina in the centre.

The refractive index distribution in the crystalline cones was revealed with a new method for computer analysis of interferograms from intact, live crystalline cones. These and ray tracing applied to the optical system revealed that the crystalline cones have the following properties:

1. The distal part contains a graded index lens, corresponding to the corneal lens in insect apposition eyes.

2. In the proximal half of the cone, a second gradient was found with an increasing refractive index towards the proximal tip. This gradient acts by rejecting off-axis rays and rays entering from adjacent cones. Thereby, the proximal gradient replaces interconal screening pigment, which is present in apposition eyes of other arthropods.

3. The distal and proximal index gradients are separated by a portion of almost uniform refractive index.

4. The most proximal part of the cone acts as a short graded index optical fibre for rays within the acceptance angle.

Because the gradient system does not seem to confer any optical advantage compared with the presence of shielding pigments, it is interpreted as an adaptation to achieve a transparent, and thus less visible eye in a basically pelagic animal.

The optical information capacity was evaluated for different regions of the eye, and the dorsal part had interommatidial angles under 1 degree.

During the preparation of the final typescript of the present paper, a description of the optics in a

similar eye of another hyperiid amphipod appeared in this journal (Land 1981). The results of these two independent investigations are partially similar, but the methods and the interpretation of the results are different, and therefore, a comparison of the two investigations is given in the Discussion.

Introduction

The family Hyperiididae comprises an aberrant group of amphipod crustaceans. They are characterized by a pelagic life, and although most species are large amphipods, they appear almost transparent.

Hyperiiids in general have large compound eyes covering most of the head. The eyes differ from other compound eyes by possessing extremely long crystalline cones that are not surrounded by pigment (Ball 1977; Meyer-Rochow 1978; Hallberg et al. 1980). Ball (1977) interpreted the structure of the eye of the hyperiid *Phronima* as an adaptation to achieve a transparent eye but how this is optically accomplished remained unknown. Because of the dense retinal pigmentation, the superposition principle (Vogt 1980; Land 1980a, b; Kunze 1979) cannot explain the pigment-free zone, and the apposition principle demands optical isolation between the ommatidia. The question how the long crystalline cones can maintain optical isolation without screening pigment can be answered only by a thorough investigation of the ray path in the crystalline cones.

From electron micrographs, regions of different density can be seen in the cones of hyperiidids (Hallberg et al. 1980), suggesting the existence of refractive index gradients. Interferometric examinations of gradient optical systems have previously been made on sections cut from fresh or fixed material (Kunze and Hausen 1971; Horridge et al. 1972, 1977; Hausen

1973; Vogt 1974; Land 1979; Land and Burton 1979; Bryceson 1981; Caveney and McIntyre 1981). However, the crystalline cones of hyperiids, as well as those of many other crustaceans, are very soft and unstable, making fresh sectioning impossible. Therefore, the present investigation included the development of a method for interferometric gradient analysis of intact crystalline cones.

Because the compound eye of *Hyperia galba* is typical of hyperiids and because this species is easily available in large numbers, it was used in this investigation.

Materials and Methods

Adult females of *Hyperia galba* were collected from the moon jellyfish (*Aurelia aurita*) on the Swedish west coast. (The males of the species are smaller and less common.) The animals were kept in aquaria maximally 2 days before examination.

Interferometric analysis was performed on free crystalline cones, carefully dissected out from light-adapted eyes with needles. Dissection was performed in a salt solution consisting of 420 mmol/l NaCl, 10 mmol/l KCl, 12 mmol/l CaCl₂, 25 mmol/l MgCl₂ and 10 mmol/l HEPES buffer in distilled water (basic solution). The most successful preparations were made after the eye had been pre-treated, for 5 min, in a modified salt solution; 5 mmol/l EDTA in the same solution as above, but without MgCl₂ and CaCl₂. This pre-treatment freed the crystalline cones from the retina and the cuticle, assuring a minimum of mechanical damage. Free crystalline cones were placed on an object slide in a drop of the basic salt solution and supplied with a cover glass fixed at a distance of 100 µm above the object slide with two 100 µm glass rods.

The preparations were examined in a Zeiss interference microscope and photographed in white light with Kodak Ektachrome 64 within 2 min after completed preparation. An additional photograph for wavelength calibration was also taken using a 546 nm interference filter. The enlarged colour photographs were superimposed with a co-ordinate net and lines were drawn perpendicular to the long axis of the cone. The phase shift was then measured along these lines by decoding the inference colours. With sufficient accuracy each quarter of a wavelength could be determined.

The phase shift profiles were transformed into refractive index profiles by a computer program (Fig. 1). This program was written in FORTRAN 77 and executed by a Univac 1100. The program was designed on the following basis: The crystalline cones were assumed to be symmetric around the optical axis. Because the beam path in the interference microscope is perpendicular to the symmetry axis (optical axis) of the crystalline cone, it was possible to apply two-dimensional ray tracing in a circular system. Therefore, a number of parallel rays (one for each phase shift value) have been traced through the system to simulate the ray path in the interference microscope (Fig. 2). Initially, each ray is traced straight and the path distance is calculated. From this and the phase shift, the refractive index is calculated and used for a new and more correct tracing with a curved path. This procedure is repeated several times (10–20) for the ray, forcing the approximated refractive index and ray path to approach the true conditions. The peripheral-most ray is traced first. Because the refractive index in the ray path through the cone is initially unknown, it is, as a first approximation, set to the refractive index of the surrounding medium (1.335). The ray will then travel in a straight line. The path distance through the system is calculated and together with the phase shift measured for the ray, the following equation will

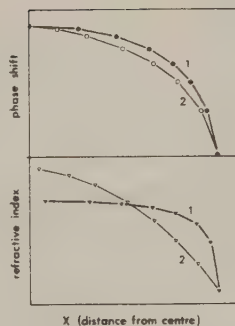


Fig. 1. Input and output graphs from the computer program. The phase shift profiles (upper graphs) differ little between homogeneous and grade-index objects. Therefore, it is difficult to see from the phase shift values what index profile they correspond to. The program will yield the same number of index values (lower graphs) as phase shift values received

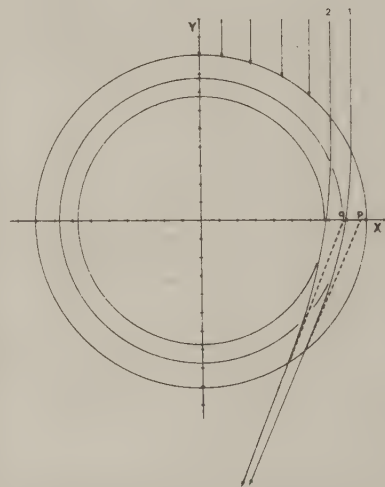


Fig. 2. Calculation geometry for the computer program. The diagram shows the ray path in the interference microscope through a plane in the cone perpendicular to its optical axis. The two first rays (1 and 2) are traced and the extrapolated intersections (*p* and *q*) with the focal plane of the microscope (*X*-axis) reveal their apparent positions. The two inner circles represent the shells through which these rays travel

give the refractive index that the ray will experience (Varela and Wäntanen 1970):

$$n_m = n_0 + \frac{\Gamma}{s_m} \quad (1)$$

where n_m is the refractive index of the object layer; n_0 , the refractive index of the surrounding medium; Γ , the phase shift; and s the path distance. The calculated refractive index constitutes the second approximation. This value is converted to a linear refractive index gradient, which gives the ray path the same average refractive index as the approximation.

The same ray is then traced again, now using the approximated gradient. Tracing through the gradient is achieved by the following equation (Megitt and Meyer-Rochow 1975):

$$r = \frac{n_1 - n_2}{\frac{\sin \theta}{\Delta S} (n_2 - n_1)} \quad (2)$$

where r is the radius of curvature of the ray path; θ , the angle between the ray and the gradient; ΔS , the distance between the two refractive indices, n_1 and n_2 , along the gradient. Since θ is continuously changing through the ray path, the tracing can only be made for a short distance before θ is changed. This integration distance is kept below 1% of the complete ray path to ensure that this discrepancy of θ never exceeds 1°. The ray curvature at each integration distance is assigned a local co-ordinate system with the zero point at the centre of the ray curvature. The change in co-ordinates, ΔX and ΔY , for each integration distance is then added to the global co-ordinates (with the zero point in the centre of the gradient curvature) before the ray is traced another integration distance.

When the ray reaches the border of the cone, it is linearly extrapolated backwards to the focal plane of the microscope (X-axis in Fig. 2). From the intersection with the X-axis, the phase shift is interpolated linearly from the measured values. This routine will reveal the observed phase shift for the ray, corrected for the focusing effect of the gradient. The new phase shift and the path length (summed integration distances) will give a new approximation of the refractive index by again using Eq. 1. The ray is then repeatedly traced, giving consecutively better values of the slope of the refractive index gradient. The retracing is terminated when two immediately following index approximations are identical within three decimals. However, one approximation is not always better than the previous one, but the two values are in that case always on different sides of the true value. To overcome this problem, which will cause the approximations to oscillate around the true value, a damping is introduced. It is achieved by using each time an average between the two latest approximations.

After ray 1 has yielded a stable value, the other rays are calculated in sequence with the same routines. All the rays, except ray 1, will travel through shells of previously established index gradients (one shell per ray). Consequently, the refractive index cannot be calculated by Eq. 1, so the following equation has been deduced

$$n_m = n_0 + \frac{\Gamma - \sum_{i=1}^{m-1} s_i(n_i - n_0)}{s_m} \quad (3)$$

where s_m is the path distance through layer m (the remaining parameters are defined in Eq. (1)).

The program is continued until all the rays from the periphery to the centre (one for each measured value of phase shift), yield a stable value. It is, of course, possible to trace more rays than

there are measured phase-shift values, but this will only add a false accuracy.

The computer program was used to calculate the refractive index profile of the left and right sides of the crystalline cones at several places along their optical axes. The refractive index profiles were combined to create refractive index models of the whole crystalline cones.

Ray tracings in the cone models were made semi-manually with a programmable calculator using Eq. 2 in a co-ordinate system.

Results

General Description of the Eye

The compound eyes of *Hyperia galba* are large transparent structures, covering most of the animal's head (Fig. 3a). About 1,150 ommatidia are present. The retina appears as a small dark patch inside the eye. The living eye displays a distinct, dark pseudopupil, comprising only a few ommatidia, when observed with a narrow aperture objective. In the lateral part of the eye, the pseudopupil is restricted to one ommatidium at a time (Fig. 3b), but it is never possible to find an angle where there are no dark ommatidia. An extensive visual overlap is observed between the two eyes, especially in the dorsal region. The ommatidial openings are circular and closely apposed to one another, leaving only very small blind areas in between.

The fine structure of the ommatidia has been described by Hallberg et al. (1980), and only the arrangement of ommatidial components will briefly be recapitulated here. The cuticle is flat and undifferentiated, and appears homogenous in the interference microscope. The inside of the cuticle is covered with a layer of hypodermal cells. The crystalline cones are extremely long and surrounded by haemolymph (Figs. 3c, 4). Of the four cone cells, only two contribute to the formation of the cone. There are no distal pigment cells between the cones, and thus the cones are the only structures outside the retina that can have any optical significance, such as refraction, reflection or absorption. The cones taper continuously towards the proximal tip, which penetrates into the retina. Brown pigment cells encapsulate the retina, as a light proof box, penetrated on one side by the cone tips and on the other by retinula cell axons. The rhabdoms are 130–150 μm long and 10–15 μm wide, with a constriction where they abut the cones. There is a well-developed palisade, suggesting that the rhabdoms have a light-guide function, as in most apposition eyes. A peculiar feature is the retinula cell processes, lying outside the retina, between the proximal parts of the crystalline cones.

There are large regional differences in the eye, which can be seen from the shape of the crystalline

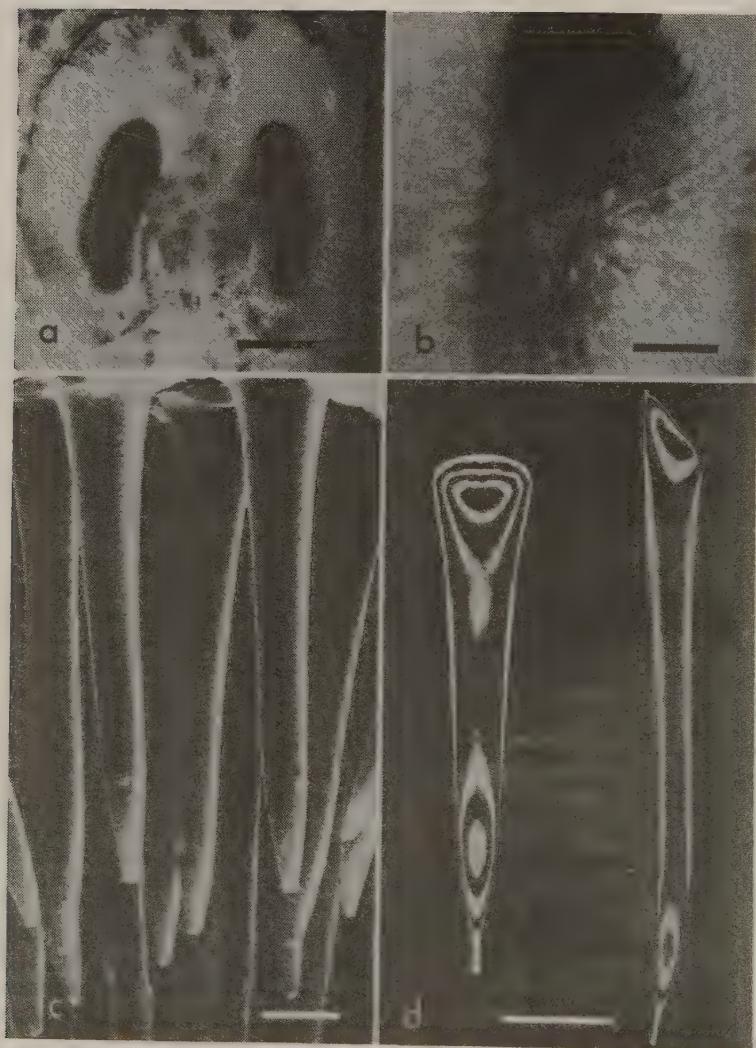


Fig. 3. a Frontal view of *Hyperia* revealing the large transparent eyes and the small dark retinae. Bar: 1 mm. b The pseudopupil in the lateral eye region observed with orthodromic illumination. Bar: 200 μ m. c The crystalline cones of the lateral region as they appear in the scanning electron microscope when the retina is detached. The shape of the cones is distorted by a pronounced shrinkage. Bar: 50 μ m. d Interference micrographs of two cones, corresponding to cone types 1 (right) and 2 (left) in Fig. 4. Cone 2 (left) is seen here from its symmetric side. The photographs are combinations from several high-magnification micrographs. Bar: 100 μ m

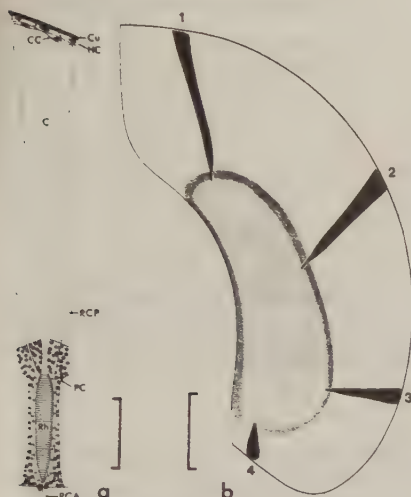


Fig. 4. a Semi-schematic representation of one ommatidium; Cu cuticle; HC hypodermal cells; C crystalline cone; CC nuclear region of cone cells; RCP retinula cell processes; PC screening pigment cell; Rh rhabdom; RCA retinula cell axons. Scale: 100 μm. b Diagrammatic representation of the eye of *Hyperia*, frontal view. Shape and position of the four selected cone types are indicated. Retina and pigmentation are represented by the stippled area. Scale: 300 μm

cones (Figs. 3d, 4b). The longest cones occur in the dorsal part of the eye (600 μm) and the length, decreases moderately towards the lateral part (350 μm). Thereafter, towards the ventral part of the eye, the length of the cones decreases dramatically (minimum, 125 μm). However, going from the rostral to the caudal part, only small differences can be observed in length and shape. The diameter of the distal endings (facet size) is largest in the dorsolateral part of the eye (about 85 μm) and smallest in the rostral part (about 45 μm). Because the retina is not in the centre of curvature of the cuticle, the cones will meet the cuticle at a certain angle, resulting in an asymmetric top on each cone. Also the shape of the proximal tip of the cone is dependent on its location. In the dorsal region of the eye the cone tip is parallel-sided (diameter, approx. 8 μm) and widens slightly where it abuts the rhabdom. Ventral to the eye's midline, the proximal cone tips never widen but continue to decrease in diameter all the way down to the proximal end.

For the optical examinations (below) four types of crystalline cones were chosen as representatives.

The shape and position of these cones are shown in Fig. 4a.

Upon aldehyde fixation the cones shrink, especially in the middle. This shrinkage becomes dramatic when the eyes are dehydrated for histological purposes. Typically, the original shape is transformed into a trumpet-like shape. A similar artefact has been observed in another hyperiid amphipod, *Streetsia challengerii* (Meyer-Rochow 1978). An effect of this shrinkage is seen on the pseudopupil of *Hyperia*, which turns into a number of widely separated spots, one for each ommatidium. This effect, which obviously deranges the optics, is seen first after 20–30 min in aldehyde fixative, even if the eye is cut open to allow free penetration. (The aldehyde fixative refers to the paraformaldehyde-glutaraldehyde solution of Karnovsky (1965)). The implications of this observation is that optical measurements have to be performed within 20 min after fixation, but preferably on fresh material. It is, however, difficult to obtain undamaged isolated cones for refractive index measurements from fresh material, but despite the low success rate, it was preferred to fixed material, which, however, is much easier to handle. The decision to use fresh material was based on the observation that fresh cones were either wholly undamaged or severely collapsed in the preparations, whereas all the fixed material displayed a slow, continuous change in shape and interference pattern.

Analysis of Refractive Index Gradients

The four representative cones chosen above were photographed in the interference microscope. The interference fringes were used to obtain phase-shift profiles at regular intervals along the axes of the cones. For cones 1, 2 and 3, 14 double profiles (left and right) were measured. For the short cone 4, 12 double profiles were taken. These profiles were analysed with the computer program described in the Materials and Methods section, to obtain the corresponding refractive index profiles.

The refractive indices from the profiles were plotted into shape models revealed by the original interference micrographs. Points with equal refractive index were connected with smooth curves, and for difficult or critical parts additional refractive index profiles were calculated with the program. It was remarkably easy to find smooth curves that fitted the values. No information could be obtained for the distal surfaces because the computer program only handles with cylindrical symmetry. Because these undefined parts of the cones constitute only about 1% of the cone length, the isoidex curves in this area were drawn to follow the shape of the interference fringes.

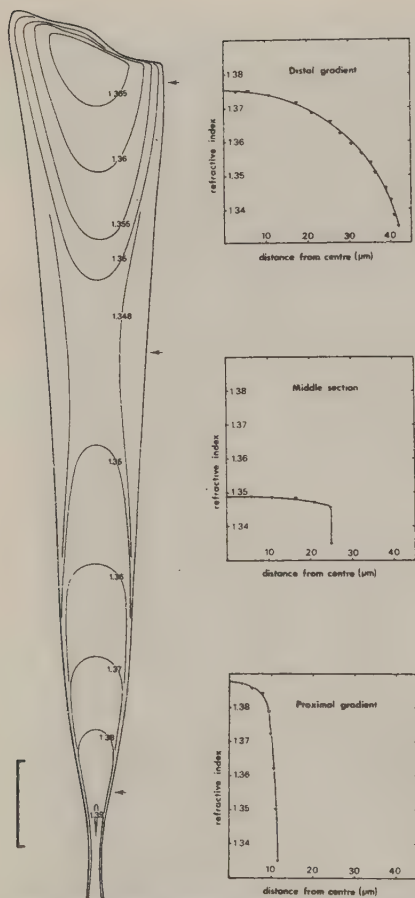


Fig. 5. Refractive index distribution in a type 2 cone. Scale: 50 μ m. The refractive index profiles at three locations are given, positions indicated by arrows

However, the possible error caused by this assumption can by no means have any observable effect on the ray path through the cones.

In principle, the four cone models all had the same type of gradients; viz. one distal and one proximal gradient, separated by a homogenous section (see cone 2 in Fig. 5). The distal gradient has a lens cylinder-like profile, with a similar shape in the four cones,

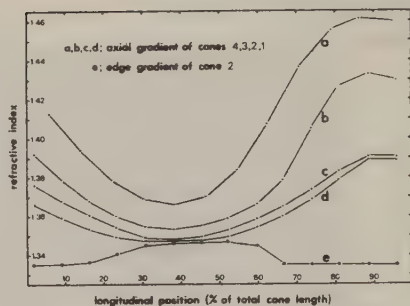


Fig. 6. Refractive index gradients along the optical axes of the four cone types and the refractive index at the edge of cone 2. The surrounding haemolymph has a refractive index of 1.335

but reaching higher axial values in the shorter ones (Fig. 6). The intermediate section is relatively longer in the longer cones. It has an almost homogenous refractive index that is considerably higher than the surrounding haemolymph, even at the cone edge. The proximal gradient is remarkable in that it has a flat-topped profile in the long cones and a more lens cylinder-like profile in the shorter cones. This gradient increases continuously proximally and reaches a maximum index value of 1.461 in the shortest cone (4) and 1.389 in the longest one (1). This gradient increase never reaches all the way down to the proximal end, but declines to form the highest index profile a short distance above the proximal end.

The refractive index of the surrounding haemolymph was measured in an Abbe refractometer and was found to be 1.335 ± 0.001 (s.d.) (average of measurements from 10 animals).

Ray Tracing

The cone models outlined above were magnified to a large scale and analysed with ray tracing in order to reveal the optical significance of the gradient systems. To begin with, only a few rays were traced at different incident angles into the models to obtain an estimate of the acceptance angles. Thereafter, three angles were chosen for each cone: (i) Effective zero degree angle. Because of the asymmetric distal surfaces, incident angles of 0.24° – 0.30° resulted in central rays parallel to the optical axes. Therefore, an angle of 0.3° was taken as an effective zero angle for all the cones, and other angles were correlated with this. (ii) An angle approaching the acceptance angle but still within it. (iii) An angle just outside the acceptance



Fig. 7a-c. Ray tracing through cone 2. Incident angles are a. 0° and b. 1.1° . Originally, nine rays were traced at each angle, but they are reduced to five in the illustration to improve the discernibility of rays in the proximal part. The pigment level is indicated by broken lines and the distal rhabdom tip has been given a striped pattern. In c, rays are incident parallel to the side of the cone to demonstrate the optical isolation. Arrows indicate starting positions of the rays

angle, to determine the destiny of non-accepted rays. The acceptance angles of the different cones are listed in the next section. Ray tracings were then performed for the three angles above, with nine equally spaced rays in each. Because the optical behaviour was very similar in all four cones, except for the different acceptance angles, only cone 2 is illustrated (Fig. 7).

The function of the distal gradient is obviously as simple as to be the focusing lens of the system. As such, it is rather good, without serious aberrations. The distal gradient receives only a little help from the proximal one to attain a focus just above the pigment level (see Fig. 7). The proximal tip acts as a short graded-index light-guide, forcing the rays

within the acceptance angle to enter the rhabdom at modest angles to the optical axes. This is a reasonable mechanism because the light otherwise would not undergo total internal reflections in the rhabdom. In the tracings just within the acceptance angle, the optical behaviour was similar to the zero degree tracings, but it was difficult to determine the positions of the foci. For incident angles just outside the acceptance angle, the rays leave the cones in the pigment-surrounded proximal region, thus being absorbed by the screening pigment.

The significance of the proximal gradient and the intermediate homogeneous part is better understood by tracing rays entering the system from the sides of the crystalline cones. Such tracings simulate light

leakage from one cone to another. The most critical situation was traced, namely, rays incident parallel to the sides of the cones. These tracings revealed that light entering the sides of the cones will fall out again at the opposite side without any possibility of reaching the rhabdom (Fig. 7c). In fact, this observation fully explains the presence of these peculiar crystalline cones; they can be optically isolated without any interconal screening pigment.

The optical significance of the double gradient cones can be summarized as follows: The distal gradient is the main lens in the system, and the function of the proximal gradient is for the rhabdom to accommodate only the facet lens of its own ommatidium.

Comparison of Optical Parameters

To fully appreciate the capacity and significance of this eye, a number of optical parameters were measured and calculated for the *Hyperia* ommatidia. Also, in order to obtain a better understanding of the regional differences, the optical parameters of all four cone types are given in Table 1. The following parameters were compared: $\Delta\rho$, the acceptance angle which was estimated from the ray tracings; $\Delta\phi$, the interommatidial angle, measured on living animals mounted in a goniometer; D , facet diameter, also measured in vivo; $\Delta\rho_A$, angular diameter of airy disc, calculated as $2.44(\lambda/D)$ (Snyder 1975), where λ is the wavelength of light, set at 500 nm; P , eye parameter calculated by $D/\sqrt{3}\Delta\phi/2$ for hexagonal arrangements of ommatidia (Snyder 1977); and d_r , receptive diameter, measured as cone diameter at focus (corresponding to rhabdom diameter in ordinary apposition eyes).

Comparisons between the acceptance angle and the interommatidial angle (Table 1) show the visual overlap by neighbouring ommatidia to be largest in the dorsal part of the eye, an observation that also can be made on the pseudopupil.

From $\Delta\rho$ and $\Delta\rho_A$ values in Table 1, it is evident that diffraction plays an important role in the dorsal ommatidia, but it has only a negligible effect on the lateral and ventral ommatidia. The same difference between ventral and dorsal ommatidia can be seen from the P values, where cone 1 comes closest to the diffraction limit ($P=0.29$ for a hexagonal lattice, Snyder 1977). These regional differences are easily understandable, because lower P values also imply optimization to higher light intensities (Snyder 1977; Snyder et al. 1977; Horridge 1978).

The receptive diameter (effective rhabdom diameter) increases towards the ventral part of the eye to about double the value. However, a corresponding increase in the anatomical rhabdom diameter cannot

Table 1. Optical parameters of the four ommatidial types in the compound eye of *Hyperia*

Cone no.	$\Delta\rho$	$\Delta\phi$	D (μm)	$\Delta\rho_A$	P (μm)	d_r (μm)
1	1.2°	0.8°	63	1.1°	0.77	12.1
2	1.9°	1.3°	84	0.8°	1.66	13.5
3	4.2°	3.5°	67	1.0°	3.26	18.0
4	13.5°	10.5°	51	1.4°	8.13	23.5

be seen from histological sections. The interpretation of this must be that the proximal tips of the shorter (ventral) cones are more efficient light concentrators owing to their higher refractive index in the proximal gradient.

Discussion

Methods Applied

Measuring refractive indices in tissue components entail a number of difficulties. First, the object to be measured must be isolated from the tissue without being damaged. This is not a serious difficulty for rigid objects, such as cuticular structures (Vogt 1974; Land 1979; Bryceson 1981; Caveney and McIntyre 1981; Nilsson and Nilsson 1981), but when dealing with the soft crystalline cones of many crustacean compound eyes, isolation is very difficult. In this investigation it was solved by the principles outlined by Nilsson and Odseius (1981), namely chemical detachment from the surrounding tissue using EDTA and immersion in a balanced, buffered salt solution.

Another problem arises if the refractive index is not homogeneous. The object beam in the interference microscope will thus travel through a continuously changing refractive index, making a straightforward calculation impossible. To overcome this problem, thin sections can be cut from the object to minimize the refractive index difference in the beam path through the object (Seitz 1969; Kunze and Hausen 1971; Horridge et al. 1972, 1977; Hausen 1973; Land and Burton 1979). Measurement on sections will yield rather good values if the sections are thin and the gradients are not too steep. However, a certain error is inevitable because the object beam in the interference microscope will be bent, causing a displacement of the interference fringes (Fölster and Herrmann 1974; Stone and Burrus 1975). Furthermore, soft tissues cannot be cut into sections.

The method described in the present investigation is based on a computerized analysis designed for measurements of intact, radially symmetric objects, such as most crystalline cones, crystalline tracts and corne-

al processes, as well as manufactured graded-index waveguides. In fact, a method for non-destructive interferometric analysis of optical fibers has been described earlier (Saunders and Gardner 1977), but their method assumed a given type of gradient, whereas the present method can handle an arbitrary gradient.

The method simulates the ray path through the object in the interference microscope and operates by repeated approximations that are continuously improved until they fit the measured values. The calculations are corrected for the curved ray path that will increase the path length and make the position of the interference fringes dependent on the focus level in the microscope. The principle enables the approximated gradient to approach any desired accuracy and the final accuracy will thus depend on the degree of symmetry in the object and the resolution of the interference pattern in the microscope. During the investigation it turned out that irregularities, caused by mechanical damage of the cones, is the only important source of error in this method. This error must be reduced by critical selection of the objects, because even small irregularities in the phase shift curves will be displayed as 'bumps' in the index profiles.

This new method opens the possibility to analyse optical systems that previously could only be explained by guesses and assumptions. It seems useful today when graded index optics is being demonstrated or suspected in the eyes of a constantly increasing number of species (see reviews: Land 1980a, b; Kunze 1979).

The Optical System of the Cone

Despite the complex refractive index gradient, the overall optical system is simple and easily understood. The distal gradient acts as the focusing lens of the ommatidium. No corneal lens is present since the cuticle is flat and optically homogeneous. The unique and powerful proximal gradient acts as an angle discriminator, which only accepts light from its 'own' distal gradient. Off-axis light and light entering from neighbouring cones cannot reach the rhabdom.

A notable effect of this system is that much of the light that is rejected by the cone will illuminate the reticular cell processes that lie just above the pigmented layer.

The shape of the refractive index profiles (Fig. 5) is different along the cone. The profile of the distal gradient is the one that comes closest to the profile of an ideal lens cylinder (see Hausen 1973; Land and Burton 1979). The intermediate section of the cone and the proximal gradient have index profiles clearly different from the ideal lens cylinder. At the edge of the intermediate section, the refractive index

is still higher than the surrounding haemolymph, which is necessary to maintain the optical isolation for light from adjacent cones. Such side-entering rays will be refracted at the edge and forced to leave the cone on the opposite side (Fig. 7c). The proximal gradient can be described as a funnel-shaped light-guide. The increased refractive index proximally must be very precise because if it is too high, or too low, even the unwanted light will reach the rhabdom. The proximal gradient in the longer cones must have a flat-topped profile to enable the paraxial rays to be focused deep in it. A more conventional lens-cylinder gradient at this place would force the rays to a more distal focus, and thus negate this optical system. This demand is not valid for the shorter cones, in which the focal length perforce must also be shorter.

Summing up the function, the proximal gradient will replace interconal screening pigment, which indeed is absent in the eye. There is no reason to believe that either one of the two solutions for optical isolation is better than the other. Therefore, the reason for the aberrant eye structure in *Hyperia* is not to be found in the visual performance of the eye. Other amphipoids, except the suborder Hyperidae to which *Hyperia* belongs, have ordinary compound eyes with screening pigment between the crystalline cones (Strauss 1926; Debaisieux 1944; Michel and Anders 1954; Hallberg et al. 1980). Evolutionarily, the *Hyperia* eye must have been derived from an eye having interconal screening pigment. The only advantage of this type of eye that might have forced its evolutionary development is its transparency.

Why Transparent Eyes?

Epibenthic animals can gain camouflage by having the same colour as the bottom substrate. However, for pelagic animals the only means or camouflage is transparency. *Hyperia* is a parasite on jellyfish, upon whose tissue it feeds (Dahl 1959a, b), but basically it is a pelagic animal that has to find its host in the open water. The body of *Hyperia* is sparsely pigmented, and together with the transparent eyes, this must be a beneficial adaptation particularly when the animal is free-swimming. Because most species of jellyfish are also transparent, the camouflage strategy may be advantageous even when *Hyperia* is feeding. This is supported by the observation that young whiting (*Gadus merlangus*) frequently search for prey in the tentacle brush of some jellyfish species (Dahl 1961).

Basically the same type of eye morphology, without pigment screened cones, has been described in several other members of the hyperiid family; *Phronima* (Ball 1977), *Streetsia* (Meyer-Rochow 1978) and

Parathemisto (Hallberg et al. 1980). *Streetsia* and *Parathemisto* are true pelagic animals and *Phronima* inhabits the transparent barrels of tunicates. Furthermore, these hyperiids share the common feature of a transparent body. It seems thus that not only *Hyperia* but the entire hyperiid family has evolved a special type of apposition eye in an effort to minimize the apparent size of the eye. This strategy must be considered of special importance for hyperiids because they all have exceptionally large compound eyes.

Regional Differences

Assuming that the acuity of the eye is determined by the interommatidial angles (Snyder 1977; Snyder et al. 1977), *Hyperia* has a strikingly high information capacity in the upper part of the visual field. An interommatidial angle under 1 degree, as in the dorsal eye region of *Hyperia* (Table 1), is fully comparable to that of diurnal insects with large eyes (see Eheim and Wehner 1972; Horridge 1978, 1980; Horridge et al. 1981; Rossel 1979). In the ventral region the acuity is 10 times less, but is still comparable to many nocturnal insects and marine crustaceans (see Meyer-Rochow 1974; Walcott 1974; Leggett and Stavenga 1981).

The regional differences, of course, reflect the distribution of light in the sea. For a pelagic animal, light from above must dominate completely. A result of the uneven distribution of light is also revealed by the eye parameter, which ranges from 0.77 to 8.13 from the dorsal to the ventral region. These values correspond to light intensities, required for optimal resolution between 'interior lighting' and 'night', according to Snyder (1977). As concluded from these values, *Hyperia* can use the full information capacity of the dorsal eye region only during the daytime and at moderate depths in the sea. However, the theory of Snyder (1977) is based on resolution of a regular striped pattern, and this might not be appropriate for the normal visual surround of *Hyperia*. To detect scyphozoans (the hosts of *Hyperia*) with the dorsal part of the eye is rather a question of seeing a dark circular object against a bright background. Such a case is treated by Horridge (1975) who demonstrated that an object can be discerned when it occupies a field of 0.065 of the acceptance angle, provided that the object makes a contrast of 100% against a homogeneous bright background. This yields an object of 0.08° for an acceptance angle of 1.2° as in the dorsal part of the *Hyperia* eye. These calculations are not immediately applicable to the case of *Hyperia* seeing scyphozoans, because the contrast of the object is much less than 100% and the background may not

always be uniform. Moreover, to discriminate scyphozoans from other objects, more than one ommatidium must be involved. If it is assumed that at least three optical axes (1.6°) across the object is needed for *Hyperia* to be able to discriminate scyphozoans from other objects, an ordinary sized scyphozoan (30 cm across) will be recognized at a distance of about 10 m.

Comparison with the Eye of *Phronima*

Recently, Land (1981) described the optics in the eye of another hyperiid amphipod, *Phronima sedentaria*. Land's investigation and the present one were carried out independently; and since the article by Land appeared during the preparation of the final typescript of the present paper, a comparison of the two investigations is presented separately here.

The methods for analysing interference micrographs differ between the two investigations. Land (1981) made no correction for the curved ray-path through the object in the interference microscope. This reduces the accuracy compared to the present method. Nevertheless, Land succeeded in analysing the optical system of *Phronima* and his results show many similarities with the present results on *Hyperia*.

Phronima has eyes that are completely divided into one dorsal and one lateral part. According to Land, the optics of the crystalline cones in these two parts is different. The lateral eye has crystalline cones with two refractive index gradients, one distal and one proximal, which was also found in *Hyperia* in the present investigation. The dorsal eye of *Phronima*, moreover, has the proximal cone tips elongated into light-guides of considerable length. A corresponding structure was found in the dorsal eye region of *Hyperia*, though it is much shorter. Thus, it appears that the optical systems of the *Phronima* and *Hyperia* eyes are modifications of the same principle.

The function of proximal gradients is interpreted quite differently in the two investigations. Land explained it as a way of obtaining a shorter focal length and thus, getting a brighter image at the distal rhabdom tip, whereas in the present paper, the proximal gradient is interpreted as an alternative to interconal screening pigment. The two interpretations do not compete and probably they are both true. The proximal gradient may act as an important second lens in short cones of this type. This would make a long crystalline cone with a short focal length, which seems reasonable because it will widen the transparent zone.

However, the optical isolation without screening pigment must be the basic reason for the existence of proximal index gradients, and I am convinced that such gradients are employed in many other species where transparency is achieved by reduction of the pigment screen.

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The compound eye of *Leptodora kindtii* (Cladocera)

An adaptation to planktonic life

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Summary. Each of the approximately 500 ommatidia in the compound eye of the cladoceran crustacean *Leptodora kindtii* has a crystalline cone consisting of five cells. Five retinula cells are also present, one of which contributes to the distal 1–2 μm of the rhabdom only; the other four retinula cells form a continuous rhabdom. Throughout the rhabdom its cross section displays two separate halves with the axis of the microvilli in one half perpendicular to that in the other (orthogonal pattern). Interferometric analysis of the refractive index of the crystalline cone revealed an inhomogeneous system with one distal and one proximal gradient. The gradient system was found to exclude rays entering from adjacent facets, thus maintaining the optical isolation. Consequently, these optics replace distal screening pigment, which is absent in the eye. The long and unscreened crystalline cones give rise to an almost transparent eye in conformity with the overall transparency of this planktonic animal.

The morphological characteristics of the eye of this species deviate from other cladoceran eyes, but the optical design closely resembles that of some pelagic marine amphipod crustaceans.

Key words: Compound eye – Optics – Fine structure – Cladocera

It was recently shown that a group of pelagic amphipods, the Hyperiidae, has a characteristically transparent compound eye (Land 1981; Nilsson 1982). Since the bodies of these animals are also transparent, this type of eye was interpreted as an adaptation to aid in the camouflage of these pelagic animals.

The water-flea species *Leptodora* (Cladocera), which is planktonic in lakes, also has a remarkably transparent body. The screening pigment in

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the eye of this species is restricted to the retina in apparently the same way as in the hyperiids. In this study the optics of the *Leptodora* eye are investigated and compared with the hyperiids.

The fine structure of the hyperiid eye was described by Ball (1977) and Hallberg et al. (1980). The structure of the *Leptodora* eye was described by Wolken and Gallik (1965), but it is considered necessary to re-examine this eye in order to determine how the transparent eye design is realized at the cellular level in cladocerans. A structural investigation may also be useful for comparison with eyes of other cladocerans. To date, only a few cladoceran eyes have been examined. Among them *Daphnia* (Güldner and Wolff 1970) and *Polyphemus* (Nilsson and Odselius 1982; Odselius and Nilsson 1982) have proven to be very different.

Materials and methods

Leptodora kindtii (Focke) was obtained from the Vomb lake in south Sweden. The eyes of the light-adapted animals were prefixed according to Karnovsky (1965) for 2 h and postfixed in 1% osmium tetroxide for 1 h. For both fixatives, a 0.2 M cacodylate buffer (pH 7.3) was used. Sections were cut with glass knives and stained in 0.5% uranyl acetate and 1% phosphotungstic acid. Grids were examined in Zeiss EM 10 and Jeol 200 CX electron microscopes.

The refractive indices of the crystalline cones were analysed using interferometry of intact live cones. The cones were isolated in a salt solution consisting of 120 mM NaCl, 5.4 mM KCl, 7 mM CaCl_2 , 2.7 mM MgSO_4 and 10 mM HEPES buffer in distilled water. The viability of the cones was tested with trypan blue (Nilsson and Odselius 1981). Free, undamaged crystalline cones were photographed in an interference microscope, and the fringe pattern was used to obtain phase-shift profiles at various places along the symmetry axes of the cones. These phase-shift profiles were converted to refractive index profiles with the computerized method described by Nilsson (1982). The index profiles were combined to make a large-scale refractive-index model of a crystalline cone. This model was analysed by semimanual raytracing in a co-ordinate system, using a calculator programmed with the equation of Meggitt and Meyer-Rochow (1975) for gradient ray tracing.

Results

Morphology

The cyclopic eye, together with its lamina and medulla, is enclosed in the end of a tube-like extension from the body (Fig. 1). It resembles a single frontal eye-stalk in which the eye is movable. In the living animal, the eye is transparent owing to the lack of screening pigment in the distal two-thirds of the ommatidia (Fig. 2). The eye is spherical and consists of roughly 500 ommatidia without regional differences in morphology, except for size differences between the anterior and posterior regions of the eye. The suture between the original arrangement of two eyes is displayed as a mirror arrangement of the ommatidia on both sides of a dorsoventral line. No unpaired ommatidia are found in the borderline. Distally, the ommatidia are separated, leaving extracellular spaces between. In electron micro-



Fig. 1. Dark-field micrograph of the anterior third of a living animal, *Leptodora kindtii*. The dark retina (*r*) is seen inside the eye. The bright area around the retina is the highly refractive proximal part of the crystalline cones. The lamina (*l*) and medulla (*m*) are connected to a thin optic nerve running caudally. Bar: 200 μ m

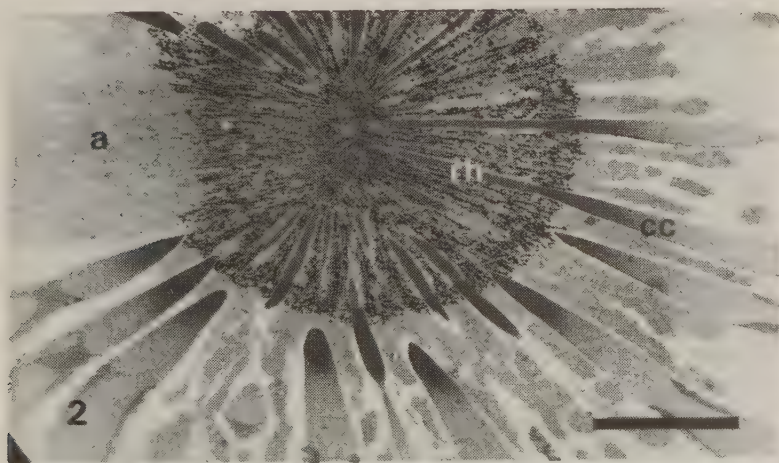


Fig. 2. Low-magnification electron micrograph of an eye. The screening pigment is restricted to the retina. The proximal electron-dense portion of the crystalline cones can be seen in contrast to the lightly stained distal portions, *rh* rhabdom; *cc* crystalline cone; *a* axons emerging from the eye. Bar: 50 μ m

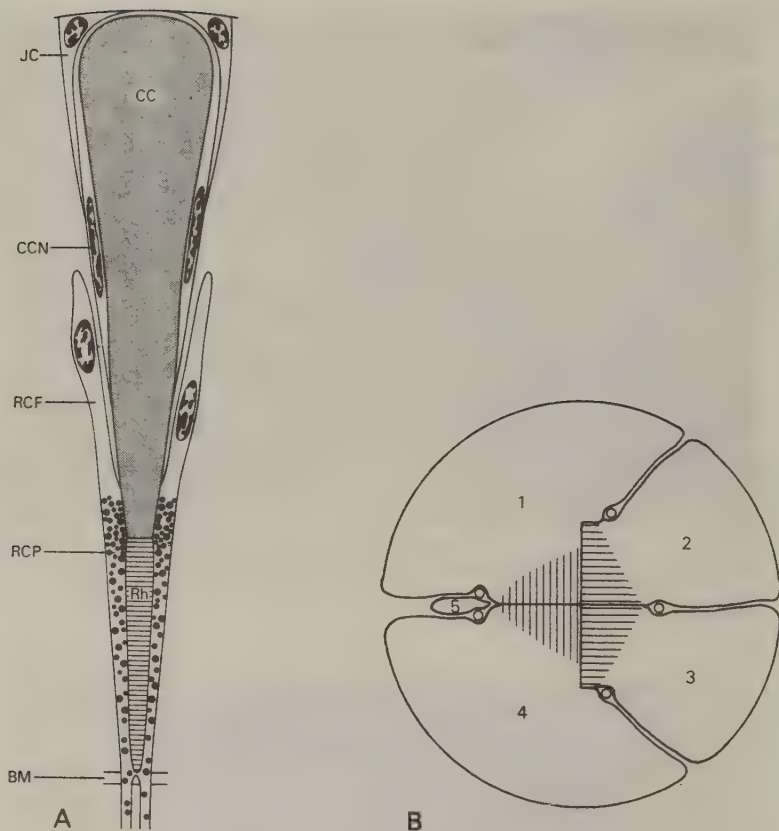


Fig. 3A, B. The ommatidial anatomy of *Leptodora*. **A** Semi-schematic drawing of an ommatidium. *JC* juxtacrystalline cell; *CC* crystalline cone; *CCN* cone cell nucleus; *RCF* retinula cell, pigment-free portion; *RCP* retinula cell, pigmented portion; *Rh* rhabdom; *BM* basement membrane. The shaded area indicates the optical part of the crystalline cone and the dots, pigment granules. **B** Schematic drawing of a cross section through the middle of a rhabdom showing the cellular arrangement and microvillar orientations. The retinula cells are numbered 1-5, of which no. 5 is aberrant (not participating in the rhabdom at this level). The cone-cell roots are represented as circles

graphs these spaces are seen to contain a flocculent material. Shrinkage of the crystalline cones during fixation and dehydration probably partly contributed to the formation of extracellular spaces.

Each ommatidium (approximately 180 μm long) has a dioptric apparatus or crystalline cone consisting of five equally large cells (Figs. 3, 4). The portions of cytoplasm containing the nuclei are confined to the distal third

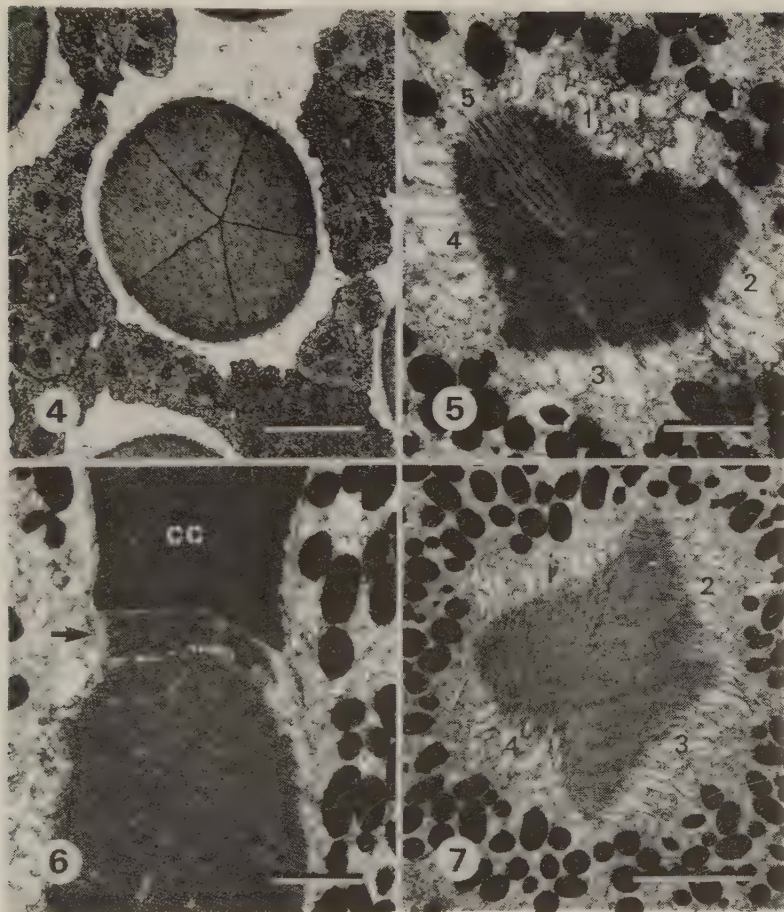


Fig. 4. Cross section through a crystalline cone composed of five cells. The cone is surrounded by pigment-free parts of retinula cells. Bar: 5 μ m

Fig. 5. Cross section through the distal extreme of the rhabdom. At this level the fifth cell contributes with a rhabdomere. Numbers in the palisade refer to the retinula cells as in Fig. 4 B. Bar: 1 μ m

Fig. 6. Longitudinal section through the junctional region between the crystalline cone (cc) and the rhabdom. The section is oblique and does not include cells 2 or 3. Arrow indicates the rhabdomere of the fifth cell. Bar: 1 μ m

Fig. 7. Cross section through the middle of the rhabdom. The four rhabdomeres are numbered in the palisade. Bar: 2 μ m

of the cone. The nuclei, situated in the periphery, are large and flat. The main (refractive) portion of the cone is 'structureless' and finely granular in appearance. Electron micrographs show a distinction in this area between a dominating light portion and a proximal dark portion. The distal end of the cone abuts the cuticle. Around the distal third of the cone two juxta-crystalline cells form a thin envelope. These cells form part of the epidermis, and their nuclei are situated around the top of the crystalline cone. The entire dioptric apparatus is approximately 100 μm in length with a distal facet diameter of 30 μm .

Five retinula cells continue the ommatidium proximally. Their distal portions surround the proximal part of the dioptric apparatus and contain the nuclei. These distal parts of the retinula cells are totally devoid of pigment granules. Pigment first appears in the cells along a sharp borderline approximately 5–15 μm above the proximal cone tip. The smaller values refer to the anterior ommatidia, the higher to the posterior. This forms a clear zone in the eye from the cuticle roughly down to the transitional zone between the cone and the rhabdom. Along the rhabdom all retinula cells are richly supplied with irregular pigment granules approximately 0.8 μm in diameter.

Four of the retinula cells form a fused continuous rhabdom. The fifth retinula cell has a small rhabdomere (less than 2 μm long), which joins the rhabdom at the distal extreme (Figs. 5, 6). A cross section of the rhabdom shows two halves with microvilli arranged perpendicular to each other (Fig. 7). One half is formed by two juxtaposed cells (2 and 3) with parallel microvilli. In the other half two cells (1 and 4) form microvilli pointing towards one another. The microvilli of the small fifth cell insert between cells 1 and 4, but are aligned with the microvilli of cells 2 and 3. Towards the basement membrane, the fifth retinula cell continues as an axon between cells 1 and 4. The microvillar pattern is thus orthogonal throughout the rhabdom, however, without being layered, which is the most common way of forming an orthogonal pattern in crustacean rhabdoms.

The rhabdom is surrounded by an extracellular palisade (see Ball 1977). It consists of the extracellular spaces formed between the stalks connecting the retinula cell body and the microvilli. Each stalk gives rise to several microvilli (Figs. 5, 7).

Each crystalline-cone cell continues as a thin strand (root) between the retinula cells immediately outside the band-desmosome sealing the rhabdom space. There is one root for each cell border, which continues to and takes part in the basement membrane; the latter also comprises an extracellular basal lamina.

At those sites where the retinula cell axons penetrate the basement membrane, there is generally no distinct arrangement in groups emerging from one ommatidium. Below the basal lamina all axons join in two tightly apposed bundles running to the lamina ganglionaris. A few groups of five axons have been found, indicating a coherence of axons from one ommatidium.

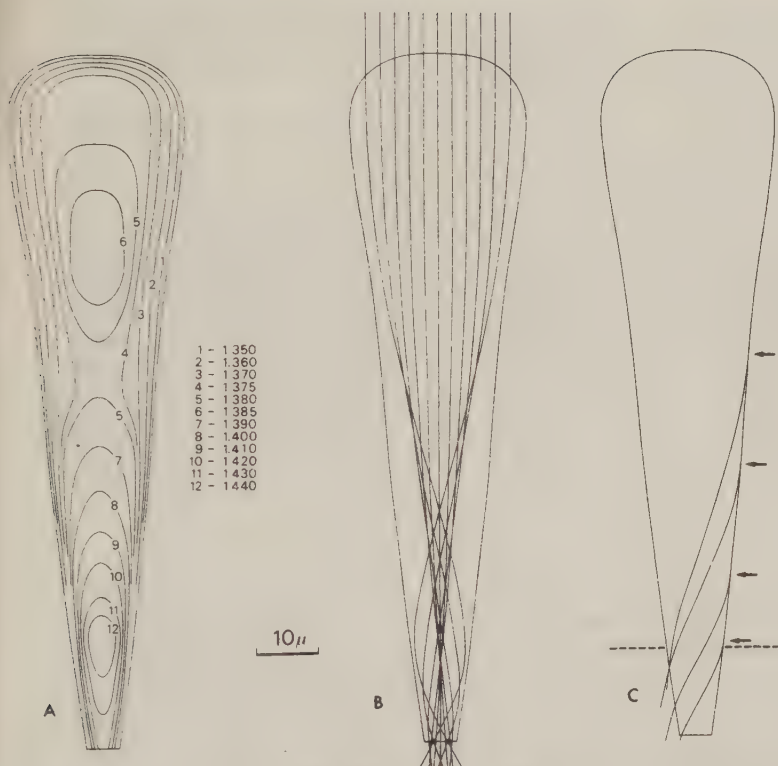


Fig. 8A-C. Optical properties of the crystalline cone. **A** Isoindex map of the refractive index gradients. Numbers are explained at the right. **B** Ray-tracing with the incident rays paraxial. **C** Ray-tracing simulating leakage of light from an adjacent cone. Four rays enter parallel to the cone border, at different positions (arrows). Dashed line indicates the extension of the pigment screen

Optics

Refractive-index analysis of the crystalline cones revealed an inhomogeneous system with two gradients (Fig. 8A). A weak gradient, reaching an axial value of 1.389, is formed in the distal part of the cone. In the proximal half, another gradient displays a remarkable pattern. Towards the proximal tip, the refractive index increases, reaching a maximum of 1.451 at the optical axis, a short distance above the proximal tip. The distal gradient has a lens-cylinder-like profile, while the proximal gradient has a more pointed profile. The two gradients are separated by a portion with a low refractive index.

Ray-tracing in this system was performed with paraxial or tilted incident rays (Fig. 8B). Parallel rays were focused slightly above the junction between the cone and rhabdom. Both gradients are responsible for focusing, but the distal one can be considered as the primary lens.

The incident beam could be tilted up to 6° and still reach the rhabdom, but at 8° all rays left the cone at the sides instead of entering the rhabdom. Assuming an acceptance limit of 7° , the complete acceptance angle would be 14° , which fits the interommatidial angle of 10° – 15° (measured from histological sections). According to the optimization theory (Snyder et al. 1977), the acceptance and interommatidial angles should be about equal.

Since there is no screening pigment around the crystalline cones, except for the most proximal portion, the optical isolation must be maintained by the gradient system. This is demonstrated by tracing rays that enter along the sides of the cone (Fig. 8C). It turns out that such rays are not accepted by the optical system. The proximal gradient forces side-entering rays to leave the cone at the side opposite to where they entered. This mechanism requires a shield of screening pigment around the proximal extreme of the cone, as is the case in *Leptodora*. As Fig. 8C shows, this screen is no larger than is necessary to maintain the optical isolation.

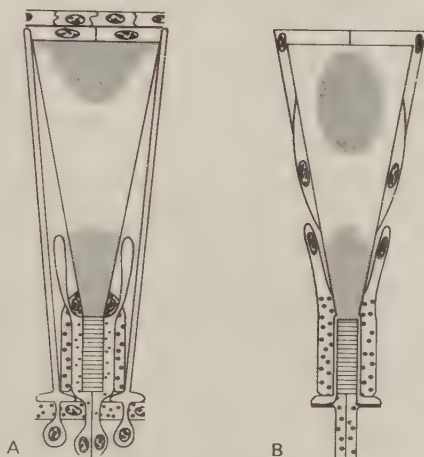
Discussion

Comparing the eye of *Leptodora* with other cladocerans, the crystalline cone composed of five cells represents the common feature (Wolken and Gallik 1965; Odselius and Nilsson 1982). The number of cone cells varies within the Crustacea (Waterman 1961; Elofsson and Odselius 1975; Hallberg 1977; Andersson 1979). Usually the number is constant for smaller taxonomic units (orders) but can also be variable within them, viz. conchostracans (Nowikoff 1905) and anostracans (Debaisieux 1944; Elofsson and Odselius 1975). The movable cyclopic compound eye, derived from paired eyes, is characteristic of both cladocerans and conchostracans.

Other morphological features of the cladoceran eye and ommatidia are highly divergent. Distal pigment cells, juxtacrystalline cells and corneagenous cells surround the crustacean crystalline cone in different combinations. The relation of the juxtacrystalline cells to the other two cell types is debated (Paulus 1979; Hallberg and Elofsson 1982). In cladocerans, the pattern varies and the juxtacrystalline cells can appear alone as in *Leptodora* or together with the distal pigment cells, as in *Polyphemus* (Odselius and Nilsson 1982).

Leptodora has five retinula cells, *Daphnia* eight (Elofsson, unpublished) and *Polyphemus*, five or six (Odselius and Nilsson 1982) depending on the region of the eye. The fifth cell in *Leptodora* and the sixth in *Polyphemus* is aberrant. The microvillar pattern of the rhabdom of *Leptodora* is unique within crustaceans; it is a non-layered orthogonal pattern. Similarly, *Daphnia* has a non-layered pattern, but the design differs greatly (Elofsson, unpublished). Still other orthogonal patterns are found in *Polyphemus* (Odselius and Nilsson 1982), but in this case there are several patterns within one eye, non-layered as well as layered.

Fig. 9. Comparison between the transparent eyes of (A) *Hyperia* (redrawn from Hallberg et al. 1980) and (B) *Leptodora*. The diagrams show how the same functional eye type has been realized with widely different cellular arrangements. Shaded areas represent the important refractive index gradients and hatched areas the rhabdoms. Pigmentation is indicated by dots. Note also the position of the nuclei



Summarizing the morphology, the ommatidial construction in the species investigated indicates that widely different evolutionary paths have been followed within the Cladocera. This is especially evident comparing the compound eye of *Leptodora* with that of *Polyphemus*. In this context it may be mentioned that recent research on sperm morphology (Wingstrand 1978) separates *Leptodora* from the other cladocerans.

The optical design and function of the ommatidia of *Leptodora*, having two refractive index gradients and lacking interconal screening pigment, is strikingly similar to the eyes of hyperiid amphipods. The eye of *Hyperia* (Nilsson 1982) and the lateral eye of *Phronima* (Land 1981) have the same optical design (Fig. 9). Nilsson (1982) suggested that this system is an adaptation, achieving an almost transparent eye to aid in camouflage. The amphipods with this type of eye are all marine pelagic predators with more or less transparent bodies. The planktonic predator *Leptodora* seems to have employed the same strategy in a fresh-water environment. Few animals of that size (10 mm) have reached a transparency so close to invisibility.

Whereas the optical systems are almost identical in *Leptodora* and the hyperiid amphipods (for the functional analysis of this eye type, see Nilsson 1982), the cellular components are arranged very differently (Fig. 9). The hyperiid crystalline cone is composed of only two cells and there are no juxtacrystalline or corneagenous cells (Hallberg et al. 1980). Furthermore, the hyperiids have accessory pigment cells, which *Leptodora* does not possess. In both cases, however, the retinula cells extend into the pigment-free zone between the crystalline cones. This can be interpreted as a result of the restricted space within the pigmented retina, which should be as small as possible to enhance transparency.

The morphological characteristics of the eye of *Leptodora* thus show an eye diverging from those of other cladocerans, whereas the function of the optic apparatus shows a convergent development compared to some

amphipod species (Fig. 9). The latter must be due to the forces governing survival in the pelagic environment.

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EYE CAMOUFLAGE IN THE ISOPOD CRUSTACEAN *ASTACILLA* *LONGICORNIS* (Sowerby)

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Abstract: The stick-like *Astacilla longicornis* (Sowerby) (Isopoda, Crustacea) is found mainly on the anthozoan *Funiculina*. The unusual development of its eyes is interpreted as a part of a refined overall camouflage. The apposition compound eyes have two functional features related to camouflaging. (1) The major part of each protruding eye-bulb is transparent owing to the lack of screening pigment around the crystalline cones. Optical isolation of the ommatidia is maintained by a proximal refractive index gradient in the crystalline cones. (2) The proximal cone tips are surrounded by a layer of reflecting pigment cells resulting in a golden hue in the depth of the eye. Probably, neither of these properties has any impact on vision. Thus concealment is the only functional explanation. Both adaptations together prevent the eyes from appearing as large dark spots on the otherwise relatively well camouflaged animal.

INTRODUCTION

The littoral isopod crustacean *Astacilla longicornis* (Sowerby) lives on sessile organisms, preferably the anthozoan *Funiculina quadrangularis* which it mimics with its long (20 mm) stick-like body (Grunner, 1965). The compound eyes protrude hemispherically from the body but, unlike other isopod eyes, they are transparent and thus do not impair the animal's camouflage. The apparent structural differences between the eyes of *Astacilla* and those of other isopods (Nemanic, 1975; H.L. Nilsson, 1982; Nilsson & Elofsson, 1977) may be explained by the optical design of the eye.

MATERIAL AND METHODS

Compound eyes of *A. longicornis* were fixed according to Karnovsky (1965), embedded in Vestopal W, sectioned (1–2 μ m thick) with glass knives, and stained with Toluidine Blue. The sections were examined employing light microscopy.

The refractive index gradients in the crystalline cones were revealed interferometrically. For this purpose, fresh cones were isolated in a salt solution consisting of 420 mM NaCl, 12 mM CaCl_2 , 25 mM MgCl_2^* , and 10 mM HEPES buffer in distilled water. Shortly after isolation, the cones were photographed in a Zeiss interference microscope and the interference patterns were analysed by the computerized method of D.-E. Nilsson (1982). A path-distance correction (Kahl & Mylin, 1965) was incorporated as described by Nilsson *et al.* (in press, a). The method converts a phase-shift profile (measured interferometrically) to a refractive index profile. Both types of

profiles are functions of the radial distance in a circular cross-section through the object. In a crystalline cone, this means that profiles are to be taken perpendicular to the long axis of the cone. In *Astacilla*, nine profiles were measured at different positions along the axis of the cone to reconstruct the gradients in the entire crystalline cone. The method is designed, however, only for continuous gradients. In discontinuous transitions (such as a refractive index difference between the immersion medium and the peripheral extreme of the object) the method yields a steep linear gradient (Nilsson *et al.*, in press, a). Thus, it is not possible to discriminate between very steep gradients and discontinuous transitions of refractive index. Because the optical consequence would be practically the same in either case, the interpretation as discontinuous transitions is preferable due to the convenience of tracing rays in such systems.

RESULTS

The compound eyes of *A. longicornis*, relatively large compared with other isopods, consist of ≈ 400 ommatidia. The hemispheric eye surface protrudes from the stick-like body, but the bulging part of the eye is transparent and difficult to discern (Fig. 1a). Also when the head is viewed laterally, the eye is well concealed. The pigmented retina, seen deep in the eye, is pale yellow. Furthermore, the pseudopupil is small and sharply demarcated, clearly indicating apposition optics (see Stavenga, 1979) (Fig. 1b).

Each ommatidium consists of a corneal lens, a long crystalline cone, and a layered rhabdom (Nilsson & Elofsson, 1977) (Figs. 1c, 2a). Dark pigment granules surround the rhabdom, mainly close to its proximal end and distally around the junction to the cone. These pigment granules are contained within the retinula cells and the accessory pigment cells (by definition, the latter cells are actually "distal pigment cells" owing to the distal position of their nuclei, see H. L. Nilsson, 1982). The proximal cone tips are surrounded by reflecting pigment cells, conferring its colour to the retina. There are no screening pigments in the distal part of the ommatidium, which is the reason for the transparency of the eye. No morphological differences were observed between light- and dark-adapted eyes.

Refractive index analysis of the crystalline cones proved gradients to be present; the profile (radial) gradients were different in the distal and proximal portions (Fig. 2b). In the distal part of the cone, the profiles displayed a weak gradient from ≈ 1.45 in the centre to ≈ 1.41 close to the peripheral border (Fig. 2b). The border was interpreted as a discontinuous interface to the surrounding extracellular fluid (refractive index ≈ 1.35 as measured on the haemolymph with an Abbe refractometer according to Nilsson & Odselius, 1981). The refractive index profiles in the proximal part of the cone were flat-topped and had a steep linear gradient at the edge (Fig. 2b). On the basis of the arguments above, the proximal profiles were considered to be homogeneous throughout.

The central value of each of the nine profiles that were analysed revealed a

EYE CAMOUFLAGE IN *ASTACILLA*

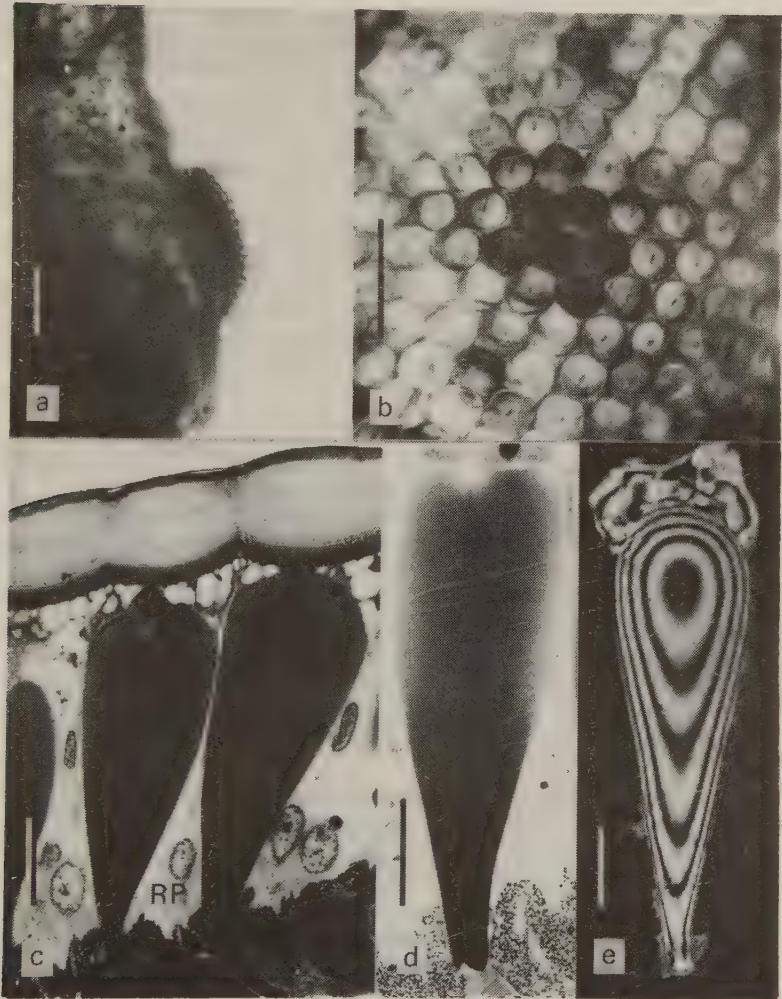


Fig. 1. a, dorsal view of the right eye of *Astacilla longicornis*: scale 300 μm . b, the pseudopupil as it appears in the middle of the eye, with orthodromic illumination (Franceschini, 1975): scale 100 μm . c, longitudinal section through the distal part of the ommatidia: note that the dark screening pigment is lacking between the cones; RP, reflecting pigment cells; scale 20 μm . d, section of a crystalline cone, stained with Toluidine Blue and illuminated with 600 nm monochromatic light: a density gradient is clearly visible; scale 20 μm . e, interference micrograph of an intact live crystalline cone: the interference pattern is a result of both the depth and refractive index of the object; scale 20 μm .

longitudinal (axial) gradient with a pronounced increase of refractive index in the proximal fourth of the cone (Fig. 2c). Combining the profiles into a two-dimensional model of the cone gave the pattern in Fig. 2a. An interference micrograph of the actual cone is shown in Fig. 1e. The calculated refractive index gradients agree well with the density gradients observed in stained sections (Fig. 1d).

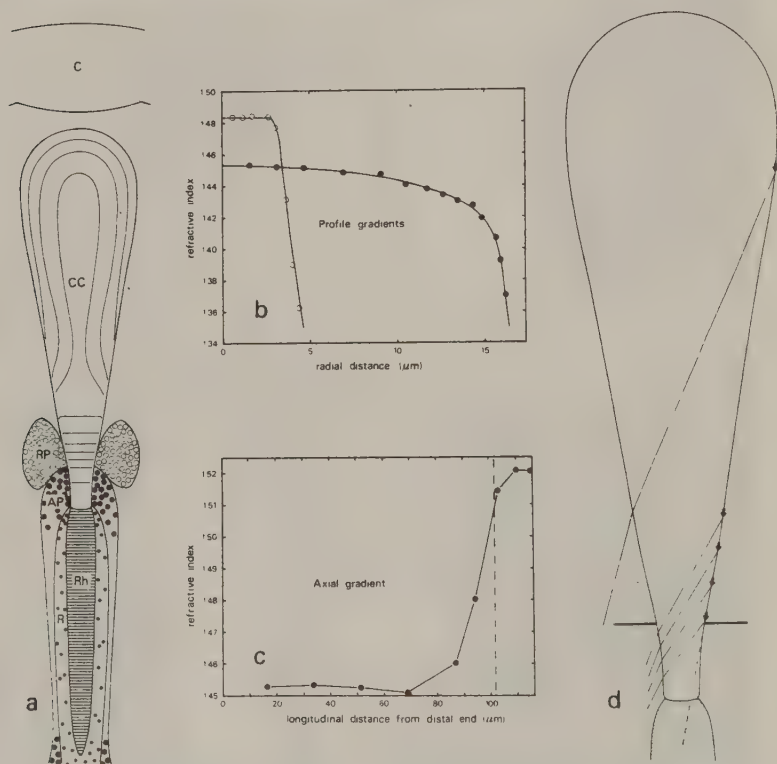


Fig. 2. a, semi-schematic drawing of an ommatidium: the refractive index gradients in the crystalline cone are drawn as isindex lines, corresponding to the following values, 1.46–1.52 (towards the proximal tip) in the proximal gradient and 1.45–1.43 (towards the periphery) in the distal gradient; C, corneal lens; CC, crystalline cone; RP, reflecting pigment cell; AP, accessory pigment cell, with absorbing pigment; R, retinula cell; Rh, rhabdom. b, profile gradients from the distal (●) and proximal (○) gradients. c, refractive index along the optical axis: the diagram represents the entire length of the cone; the dashed line indicates the distal extension of the dark screening pigment. d, ray-tracing revealing the trajectory of rays entering from an adjacent facet: the most critical case is shown, with the incident rays parallel to the border; it is obvious that no light can reach the rhabdom from adjacent facets; the distal extreme of the dark pigment screen is indicated by the horizontal line; dashed line is the straight extension of the incident rays (disregarding refraction in the cone).

Three focusing structures can be distinguished (Fig. 2a): (1) the corneal lens; (2) the distal curvature of the cone; and (3) the radial gradient in the distal part of the cone. Owing to the flat profile, the proximal gradient will make no contribution to the focusing on the rhabdom tip.

Because the pseudopupil indicated apposition optics, the ommatidia must be optically isolated. This is commonly accomplished by screening pigment, which encompasses each ommatidium throughout its entire length. In *Astacilla*, however, almost the entire crystalline cone is naked, without any pigment screen. In this region, optical isolation must be maintained by deflection of light entering from adjacent facets. The presence of such a mechanism was demonstrated by tracing rays entering along and parallel to the sides of the cone (this is the most critical case of side-entering light) (Fig. 2d). The high refractive index in the proximal part of the cone will bend rays entering from the side away from the rhabdom. Rays entering the proximal part of the cone will have to be bent more than rays entering distally, thus explaining the proximal increase in refractive index.

DISCUSSION

The compound eyes of *Astacilla* displays two unusual features; the absence of screening pigment around the cones and the presence of a proximal refractive index gradient in the cones. These two features are related in the sense that the proximal gradient replaces screening pigment as the optically isolating structure. To our knowledge, all other isopods maintain optical isolation with screening pigment between the cones (Edwards, 1969; Nemanic, 1975; H. L. Nilsson, 1982). The two alternative principles for maintaining optically isolated ommatidia are equally effective but the proximal gradient seems to be a more complicated solution. The probable reason for the deviant optical design in *Astacilla* is the need for a maximally transparent, and thus concealed eye.

Transparent compound eyes with proximal gradients in the cones occur in two other crustacean groups; in the hyperiid amphipods (Land, 1981; D.-E. Nilsson, 1982) and in the cladoceran *Leptodora kindtii* (Nilsson *et al.*, in press, b). These animals are pelagic, have transparent bodies, and the transparent eyes probably enhance camouflage. The eyes of the hyperiids (*Hyperia* and *Phronima*) and *Leptodora* differ slightly from those of *Astacilla* in having proximal refractive gradients with focusing properties.

Another feature of the eye of *Astacilla* that seems to be involved in camouflaging is the layer of reflecting pigment cells that imparts a golden hue to the eye. The light path in the cones is not affected by the reflecting pigment and, therefore, concealment seems to be the only functional explanation even in this case.

The body of *Astacilla* is stick-like, making it hard to discriminate as an animal when it resides on the anthozoan *Funiculina quadrangularis* (Schieke, 1973). Under these circumstances, the animal's camouflage would be impaired if the eyes were large, dark, and bulging structures. The special optical design, therefore, probably reduces the threat from predators with good vision and which use their eyes in detecting prey.

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THE EVOLUTIONARY LINKS BETWEEN APPPOSITION AND SUPERPOSITION OPTICS IN CRUSTACEAN EYES

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The two fundamental principles of image formation in compound eyes, apposition and superposition, have long been known to exist in both insects and crustaceans (1). The scattered distribution of the two types (2,3,4) indicates that one type can evolve into the other. However, the two optical mechanisms are so fundamentally different that it has been an enigma how such an evolution could occur. In crustaceans, superposition eyes are present among the orders Mysidacea, Euphausiacea and Decapoda (5). It is now found that the planktonic larvae of most euphausiids and decapods possess a transparent type of apposition eye, designed for planktonic life, that is pre-adapted for superposition optics. This discovery suggests that the evolution of superposition eyes in crustaceans has its origin in the special apposition optics found in the larval eyes, thus providing the first evidence of an evolutionary link between apposition and superposition optics.

The two basic types of arthropod compound eyes, apposition and superposition, employ widely different optical principles (1,5). The apposition eye has optically isolated units (ommatidia), each with a lens forming an inverted image (Fig. 1a). In superposition eyes, the lens systems of many ommatidia cooperate to form a superimposed erect image. The crucial feature of a superposition eye is for the lens system in each ommatidium to direct light towards the retina (rhabdom layer), at the same side of the ommatidial axis as it entered the eye, as shown in Fig. 1. This can be accomplished either by refraction in a lens-cylinder gradient (1,6) (Fig. 1b) or by reflections in an orthogonal system of mirrors (7,8,9) (Fig. 1c), resulting in the distinction between refracting and reflecting superposition eyes.

Both apposition and superposition eyes are present in crustaceans as well as in insects (2,3,4) but the reflecting superposition type is reported from crustaceans only (5,9). In crustaceans, superposition eyes exist in mysids, euphausiids and decapods but apposition eyes also occur within these groups (4,5) (Table 1).

The apposition principle is a simple and straight-forward one that can be traced back, in its most primitive form, to annelid worms (3). The superposition principle is more complex, and superior to apposition due to the improved light-gathering capacity (3). As based on this argument and on the scattered occurrence of superposition eyes, it seems reasonable to believe that superposition eyes have been derived from apposition eyes. However, the problem has been to understand the courses of such an evolution. No hypotheses

have been presented on this matter, simply because intermediate types between the two discrete principles were not known. Also, due to the conflict between forming erect and inverted images, it was hard to imagine the design of transitional states.

An investigation, presented here, of the larval apposition eyes of euphausiids and decapods proved to partly solve the problem by revealing functional links between apposition and superposition.

For an optical investigation, the furcilia III larva of the euphausiid Thysanoessa raschii (M. Sars) and the 11'th stage larva (megalopa) of the decapod prawn Macrobrachium rosenbergii (de Man) were chosen. These species have, as adults, refracting and reflecting superposition eyes respectively, but the planktonic larvae have 'transparent' apposition eyes without any pigment screen around the crystalline cones. For refractive index measurements, fresh crystalline cones were isolated in a salt solution of 420 mM NaCl, 25 mM MgCl₂, 12 mM CaCl₂, 10 mM KCl, and 10 mM HEPES buffer. Microinterferometry was applied and the interference patterns of intact cones were analysed by the method of Nilsson et al. (10) to determine the refractive index distribution. The focal length of the corneal lenses was measured on clean pieces of cornea, according to the method of Bryceson (11). Based on these data, ommatidial models were constructed and analysed by ray-tracing (12) (Fig. 2).

The euphausiid (Thysanoessa) larva displayed a refractive index gradient in the proximal part of the crystalline cone. An image is focused within this gradient, about half-way along its length, and the proximal part of it acts as a short graded-index light-guide, propagating light from its own corneal lens to the rhabdom. Rays from adjacent corneal lenses are rejected by the proximal gradient (Fig. 2a), thus compensating for the lack of screening pigment along the crystalline cones. This optical system is almost identical with the one found in some pelagic amphipods where the transparency achieved subserves the camouflage (13). Although it is an apposition eye (having optically isolated ommatidia and lacking the clear zone), superposition rays will be formed as an unutilized secondary effect of the proximal gradient (Fig. 2b). The resulting afocal ray-path is in principle that of a refracting superposition eye (Fig. 1) (which is realized in the adult).

In the larval eye of the decapod prawn (Macrobrachium), a distant source is focused on the rhabdom tip (as in a 'conventional' apposition eye), but also in this case, optical isolation is maintained without screening pigment between the cones. This is achieved simply by a high refractive index in the crystalline cones which deflects light, entering from adjacent facets, away from the rhabdom (Fig. 2c). The same mechanism was recently reported to result in less discernible eyes in the isopod Astacilla (14). As a result of the high refractive index, total internal reflections will occur (Fig. 2d), which provide the basis for reflecting superposition optics (Fig. 1) (which is known

to exist in the adult (15)), but as the ommatidia are optically isolated (apposition type) in the larva, superposition rays cannot be utilized for vision.

Histological sections from a number of other euphausiid and decapod larvae were examined by light microscopy. Due to the absence of screening pigment between the crystalline cones, all eyes were superficially similar. However, two groups could be distinguished, closely resembling either the Thysanoessa type with a dense rounded structure in the proximal part of the cone (representing the gradient in Fig. 2 a,b) or the Macrobrachium type lacking this structure. The distribution presented in Table 2 suggests that the optical findings in the Thysanoessa and Macrobrachium larvae are general for euphausiid and decapod larvae, respectively. However, among euphausiid larvae there is one known exception and that is Thysanopoda tricuspidata which has superposition eyes from the first furcilia instar (Land pers. comm., 16).

It is common that planktonic organisms are unpigmented because this is the only means of camouflage in their habitat. Therefore, the most probable reason for the special optical design of the apposition eyes of euphausiid and decapod larvae is to reduce the extension of the pigment screen, and hence, to make the eyes maximally transparent. For this purpose, euphausiid and decapod larvae employ two different optical mechanisms (both known from other crustaceans (13, 14, 17, 18)), providing the functional bases for the superposition principles employed by the adults. The most important changes required for the larval eyes to convert to superposition type is the creation of a clear zone (2,5) distal to the rhabdom layer, and additionally for the reflecting type, a transformation from hexagonal to square facets (9).

The discovery, in euphausiid and decapod larvae, of apposition eyes being pre-adapted for superposition optics, provides the first evidence of a possible transformation of an apposition eye into a superposition eye during evolution. There are two different mechanisms by which superposition rays are formed (but not utilized) in the larval eyes (Fig. 2), and these mechanisms are retained and fully exploited by the adults (Fig. 1). Thus, euphausiids have refracting superposition eyes and decapods reflecting ones. Not all adult decapods have superposition eyes (Table 1) but with the potential of the larval eyes to form superposition eyes, these may have evolved several times within the group.

Recently, Fincham (4) and Land (16) proposed that the different eye types in crustaceans should be important indicators of taxonomic relationship, a suggestion that must now be taken into closer consideration, since apparently, functional aspects of ontogenetic development have brought new views on evolutionary relations between eye types.

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Table 1. Distribution of eye types in mysids, euphausiids and decapods

Group	Eye type
Mysidacea	Refract. sup.
Euphausiacea adult	Refract. sup.
Euphausiacea larvae*	Apposition
Decapoda adult:	
Natantia	Reflect. sup.
Macrura	Reflect. sup.
Anomura (some)	Reflect. sup.
Anomura (other)	Apposition
Brachyura	Apposition
Decapoda larvae	Apposition

Refract. sup., refracting superposition; Reflect. sup., reflecting superposition. *) Late instars have refracting superposition eyes.

Table 2. Morphological classification of larval eyes in euphausiids and decapods

Species	Eye type
Euphausiacea:	
<u>Meganyctiphanes norvegica</u>	T
<u>Stylocheiron</u> sp.	T
<u>Euphausia</u> sp.	T
Decapoda:	
<u>Sergestes</u> sp.	M
<u>Homarus vulgaris</u>	M
<u>Galathea strigosa</u>	M
<u>Pagurus</u> sp.*	M
<u>Carcinus maenas</u> *	M

All observations are from histological sections. T, Thysanoessa type; M, Macrobrachium type. The euphausiids are early furcilia instars. The five decapod species correspond to the five decapod groups in Table 1. Mysids are not included due to the lack of free-living larval stages. *) Species with apposition eyes also in the adult stage.

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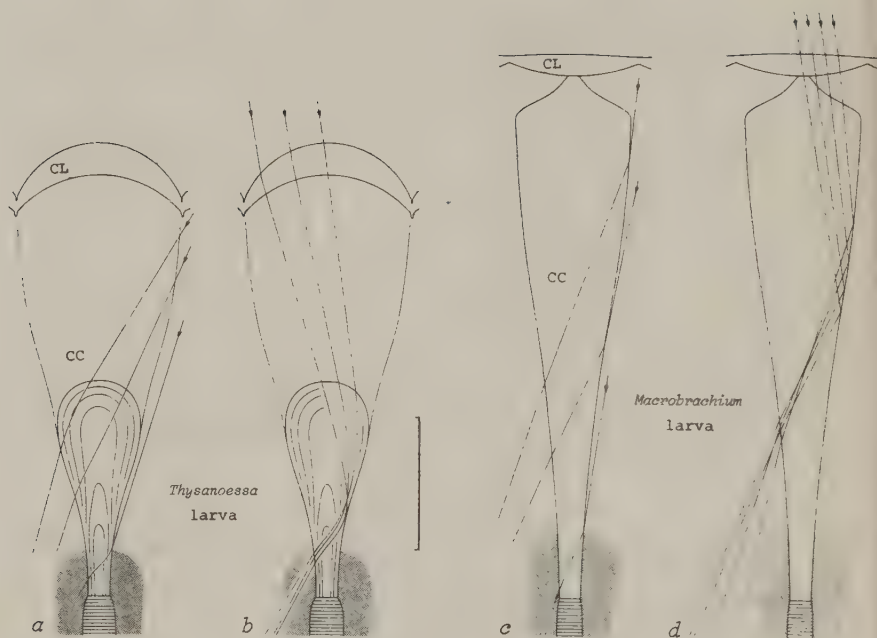


Fig. 2 Ray-tracing in ommatidial models of *Thysanoessa* and *Macrobrachium* larvae. Optical isolation (a,c) is demonstrated by side-entering rays. The formation of superposition rays, incident at 90° to the axes, is shown (b,d) (absorption in screening pigment disregarded). The proximal gradient in the crystalline cone of *Thysanoessa* (a,b) is represented by the following isoindex lines: 1.37, 1.41, 1.45, 1.49, 1.53, and 1.57, from the distal periphery to the proximal centre. The crystalline cone of *Macrobrachium* (c,d) is almost homogeneous ($n = 1.396$, with a slight increase to 1.405 in the proximal tip) and surrounded by extracellular fluid ($n = 1.339$ (15)). CL, corneal lens; CC, crystalline cone; Shaded area, screening pigment; Striped pattern, rhabdom tips; Scales, 20 μm .

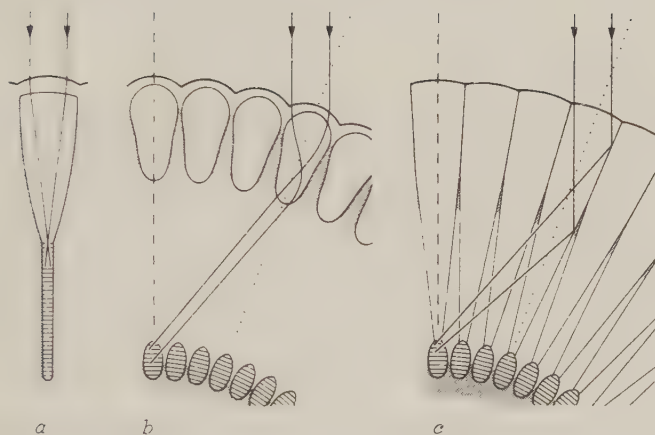


Fig. 1 Schematic drawings of the optical systems in compound eyes. In the ommatidium of the apposition eye (a), the crystalline cone is contiguous with the rhabdom (striped pattern) and parallel light is focused on the rhabdom tip. Due to the pigment screen (shaded), the ommatidia are optically isolated from their neighbours. In the superposition eyes (b,c), the clear zone distal to the rhabdoms enables light to pass across the ommatidia to form superposition images, either by refraction (b) or reflection (c). Parallel rays incident obliquely on ommatidia in b and c are projected on the rhabdom of the ommatidium which is aligned with the light source (dashed line).

Refractive Index Gradients Subserve Optical Isolation in a Light-Adapted Reflecting Superposition Eye

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ABSTRACT The freshwater shrimp *Macrobrachium rosenbergii* (de Man) has reflecting superposition compound eyes. Upon adaptation from dark to light conditions, the eye converts from superposition to apposition optics by screening pigment migration. The clear zone, typical of dark-adapted superposition eyes, persisted after adaptation to 500 lux, when the eye optically behaved like an apposition eye. In this state, optical isolation is maintained by a proximal refractive-index gradient in the crystalline cone, which only requires the proximal pigment to be in the light-adapted position around the junction between the cone and rhabdom. Thus, the distal pigment need not invade the clear zone for the eye to convert to apposition optics. This mechanism probably decreases the time required for light adaptation. Furthermore, as a result of total internal reflections in the cone tip, the proximal gradient increases the acceptance angle.

Superposition compound eyes relying on reflections have been known to exist since the report of Vogt ('75). Later investigations have provided more details about this principle, which most macruran decapods (Crustacea) seem to employ (Vogt, '77, '80; Land, '76, '78).

The reflecting superposition eye of the crayfish displays extensive pigment migration during adaptation (Kleinholz, '61; Olivo and Larsen, '78; Vogt, '80), and it is generally believed that many eyes of this type are transformed to apposition eyes during light adaptation (Land, '81a).

The present investigation was initiated to explain two contradictory observations in the light-adapted (500 lux) reflecting superposition eye of the shrimp *Macrobrachium rosenbergii* (de Man): 1) a small, typical apposition pseudopupil, and 2) a persisting, wide clear zone above the rhabdom layer. The germane question was, how could the optical isolation be maintained with a seemingly insufficient pigment screen? The problem was approached by refractive index analysis to design a two-dimensional model for testing the optical system.

MATERIAL AND METHODS

Specimens of the freshwater shrimp *Macrobrachium rosenbergii* (de Man) were obtained

from the Blentarp Aquatic Laboratory (Blentarp, Sweden). Animals with a body length of 17–20 mm were used for the investigation. The animals were kept under a daily light regime (LD 12:12). Light-adapted eyes (500 and 50,000 lux) were examined in the middle of the light period, and dark-adapted eyes in the middle of the dark period (fixation in complete darkness).

The pigment position was determined from histological sections. (The fixation was carried out in cacodylate buffer with 3% glutaraldehyde and the embedding in Epon.)

Pseudopupil observations were made on fresh eyes or eyes fixed as above. The eyes were immersed in water and viewed using a narrow aperture objective and orthodromic illumination (Franceschini, '75).

The focal length of the corneal facets was determined on cleaned pieces of cornea by microscope focusing according to the method of Bryceson ('81). The immersion medium on the outside was water, and on the inside an albumin-Ringer solution ($n_D^{20} = 1.385$) was used.

The refractive index of the crystalline cones was determined by interference microscopy. Fresh cones could not be used for this purpose because of their softness and instability. Thus, fixed cones were used (fixation as described above). Before the cones were measured, they

were transferred to a Ringer solution (Van Harreveld, '36). The fixation was judged not to alter the refractive index distribution, for neither the pseudopupil (light-adapted) nor the eye glow (dark-adapted) was affected by fixation. Interferometry was conducted with a Zeiss interference microscope using Ehringhaus or Sénarmont compensation. The refractive index of the hemolymph was measured in an Abbe refractometer.

RESULTS

Structurally, the eye of *Macrobrachium* is of the reflecting superposition type (Fig. 1). The facets are square and the crystalline cones are divided into a distal part—probably provided with a multilayer reflective coating (interference colors were seen at certain angles in dissected eyes)—and a proximal tail that tapers continuously toward the rhabdom. The entire crystalline cone is square in cross section except for the most proximal part of the tail, where the crystalline cone is circular in cross section. The retina consists of typical superposition rhabdoms: short, wide, and tightly apposed.

The interommatidial angles, $\Delta\phi$, were measured in intact eyes with a goniometer (Horrige, '78; Nilsson and Odselius, '82) and compared with measurements on histological sections. Both methods yielded $\Delta\phi$ values of about 3.7° in the center of the eye.

Pseudopupil observations revealed large differences between the light- and dark-adapted states. The dark-adapted eye displayed a yellow eye glow, with a diameter of about 12 facets. During light adaptation, the eye glow transformed into a small black pseudopupil, 2–3 facets in diameter (identical results were obtained from adaptations to 500 and 50,000 lux). Thus, there are indications that the eye converts from superposition to apposition optics during light adaptation.

The change in the pseudopupil is due to migration of screening pigment in the eye. In the dark-adapted eye, the distal pigment is confined to the space between the distal parts of the crystalline cones (Fig. 1), whereas proximal pigment (in the retinula cells) is present only around the proximalmost portion of the rhabdom. The separation of these two pigment screens creates a clear zone, enabling superposition images to be formed. During light adaptation to 500 lux, the two pigment screens moved toward each other, partly invading the clear zone. In the 500-lux-adapted eye, the clear zone was about half as wide as that of the dark-adapted eye. Adaptation to higher light in-

tensities (50,000 lux) resulted in a further reduction of the clear zone, while the appearance and size of the pseudopupil remained the same as in the eye adapted to 500 lux. The persisting clear zone in the 500-lux eyes, together with the apposition-type pseudopupil required an explanation. Therefore, the optical properties of the eye were investigated.

Interference microscopy proved that corneal lenses were present. The back focal length of these lenses was found to be $210\text{ }\mu\text{m}$ using the method of Bryceson ('81). Because the distance between cornea and rhabdoms is about $200\text{ }\mu\text{m}$, the lenses could be considered to be focused at the distal part of the rhabdoms, which accords with the findings of Bryceson ('81).

The refractive index distribution of the crystalline cones was determined interferometrically. The distal part displayed gradients, as well as discontinuous transitions in refractive index (Fig. 1). The proximal tail had a high refractive index distally and proximally and a lower refractive index in between (Fig. 2). There were no radial gradients. In the square portion this could be determined immediately in the interference microscope, but in the proximalmost circular portion, Sénarmont compensation, together with the method of Nilsson ('82), was used. There were no indications of any change in refractive index during adaptation. The hemolymph yielded refractive index values between 1.339 and 1.342 (eight measurements from different animals).

To evaluate the measured distribution of refractive index, an ommatidial model was constructed (Fig. 1). The evaluation was focused on optical phenomena in the proximal tail of the crystalline cone, for this is the only significant structure in the clear zone.

To obtain superposition imaging for all the rays, a gradient is required in the cone tail. The theoretical minimum curve for this gradient was determined for a crayfish by Vogt ('77, '80). The corresponding curve for *Macrobrachium* is plotted in Figure 2 (curve a).

In the proximal third of the tail, the refractive index rises and deviates significantly from the ideal values. Hence, superposition imaging will not occur for rays close to the optical axis. One function of the proximal gradient is revealed in Figure 3. It is evident that rays emerging from one ommatidium in the light-adapted eye cannot enter the rhabdom of another ommatidium, because such rays will be refracted in the cone tip and will be forced to leave the cone on the opposite side. The minimum refractive index required for this mech-

anism was calculated by Snell's law, and the resulting curve is plotted in Figure 2 (curve c). It can be seen that the measured values exceed the theoretical minimum curve down to the level of the proximal pigment screen. This mechanism seems to be the one that we are seeking because the ommatidia in the 500-lux light-adapted eye will be optically isolated despite the persisting clear zone.

However, the difference in distal pigment position between the 500-lux eyes and the 50,000-lux eyes remain unexplained. Because the optical isolation seems to be maintained in both of these adaptational states, it is tempting to assume changes in the acceptance angle. The ray-tracing in Figure 4 reveals the possibility of such a mechanism. If no screening pigment is in immediate contact with the cone tail, total internal reflections will increase the acceptance angle to about $1\Delta\phi$ (the incident angle is equal to half the interommatidial angle $\Delta\phi = 3.7^\circ$). This requires the possibility of two total internal reflections of the rays in the model plane. Such reflections will be prevented, or at least reduced, if the distal pigment screen lines the outside of the cone tail at the levels where these reflections occur. This type of action of the screening pigment is known to exist in many insect eyes (Franceschini and Kirschfeld, '76; Stavenga and Kuiper, '77; Stavenga et al., '77). If all reflections were eliminated, the acceptance angle could be calculated on the basis of the distal rhabdom diameter (Snyder, '77), which yields an acceptance angle of 1° . The difference between this angle and the much larger value revealed by tracing rays is due to the additional light gathering of the cone tail. From the present data, it was not possible to determine the actual acceptance angle, but it should lie between 1° (no additional light gathering) and 3.7° (maximum additional light gathering), and it is likely to be adjustable within this range by migration of the distal pigment. The pseudopupil corresponds to the larger value; but this is an unreliable indication, because the periphery of the pseudopupil may originate from absorption in screening pigment (Stavenga, '79).

DISCUSSION

The refractive index distribution in the crystalline cone of *Macrobrachium* generally accords with the findings in the crayfish *Astacus leptodactylus* (Vogt, '80), except for the increased refractive index in the proximal part of the cone tail. However, the proximal part of the tail cannot interfere significantly with the

formation of superposition images, for the cross-section area of the cone tip is only about 7% of the rhabdom cross-section area.

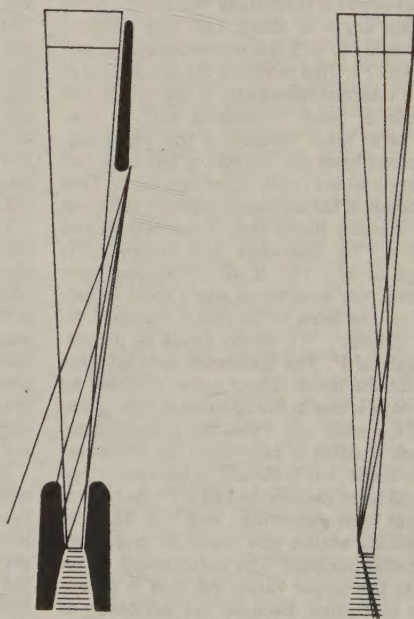
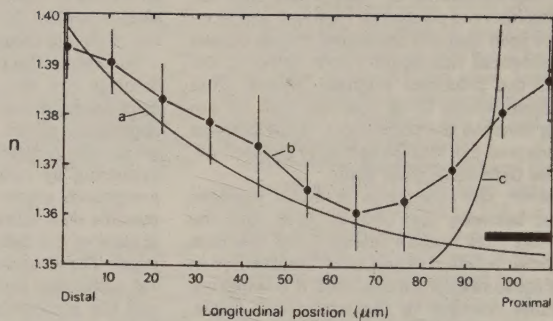
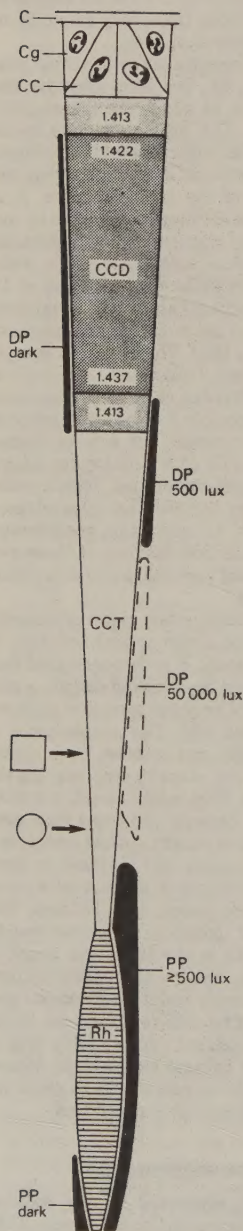
To explain the presence of the proximal gradient in the cone tail, we must consider the light-adapted state, in which the eye employs apposition optics. The high refractive index proximally in the tail will cause additional light gathering by reflections and thus widen the acceptance angle of the apposition eye. It is possible that this widening is regulated by migration of the distal pigment, as a longitudinal pupil. The theoretical acceptance angle without additional light gathering is $0.27\Delta\phi = 1^\circ$, and the corresponding value with maximum light gathering is $1\Delta\phi = 3.7^\circ$ (distal pigment retreated from the tail). The optimum acceptance angle is between $0.62\Delta\phi$ and $1.06\Delta\phi$ (Götz, '65; Snyder, '77; Snyder et al., '77; Horridge, '78). Thus, it is possible for the light-adapted eye to be at an optimum, but also to change the acceptance angles as a strategy for adapting to different light intensities. This hypothesis is supported by the fact that eyes adapted to 50,000 lux have an apposition pseudopupil like eyes adapted to 500 lux; but at these two intensities, the distal pigment is found in widely different positions.

The most interesting effect of the proximally increasing gradient is the optical isolation in the light-adapted state. It is demonstrated that optical isolation can be achieved despite a persisting clear zone if only the proximal pigment surrounds the cone tips. The consequence of this is that the eye can convert from superposition to apposition state by only moving the proximal pigment. This might result in a more rapid adaptation, because the clear zone need not be eliminated to obtain optical isolation.

This new mechanism has features in common with the transparent apposition eyes of hyperiid amphipods (Land, '81b; Nilsson, '82), where the optical isolation is maintained by proximal gradients in the cone tips, much in the same way as in the eye of *Macrobrachium*. However, the optically isolating proximal gradients are present for different reasons: In the hyperiids, a transparent eye probably aids in camouflaging the pelagic animals; in *Macrobrachium*, the mechanism seems to serve adaptation to changing light intensities.

ACKNOWLEDGMENTS

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Fig. 1. Ommatidial model from the central part of the eye of *Macrobrachium*. The refractive indexes of the distal part of the cone are indicated at the appropriate places. The refractive index of the ambient extracellular fluid was set to 1.34. The index gradient in the cone tail is plotted in Figure 2. The position where the cone tail changes from a square to a circular cross section is indicated by arrows. The position of the pigment screens is also given for the different adaptive states. Abbreviations: C, cornea; Cg, corneagenous cell; CC, crystalline cone cells (nuclear portion); CCD, distal part of crystalline cone; CCT, crystalline-cone tail; Rh, rhabdom; DP, distal pigment; PP, proximal pigment.

Fig. 2. Refractive index of the cone tail as a function of the longitudinal position (the scale also applies to Figs. 1, 3, and 4). a) Theoretical minimum curve required for superposition reflection of all rays. b) Measured gradient in

the cone tail of *Macrobrachium*. Vertical bars are standard errors from 18 measurements. c) Theoretical minimum curve for optical isolation with a persisting clear zone. It can be seen that the measured values exceed the theoretical curve down to the level of the proximal pigment in the 500-lux-adapted eye (horizontal bar).

Fig. 3. Ray path illustrating the optical isolation in eyes adapted to 500 lux. It can be seen that the rhabdom will not accept rays from adjacent facets.

Fig. 4. Ray tracing with the incident angle equal to half the interommatidial angle. The high refractive index of the cone tail confers additional light gathering by reflections. Light extraction by screening pigment is not taken into account.

